Journal of Applied Biology & Biotechnology Vol. 13(Suppl 1), pp. 62-68, July-August, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2025.214212

Effect of *Phytophthora drechsleri* f. sp *cajani* infection on local pigeon pea variety of Gujarat

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ARTICLE INFO

Article history:
Received on: 04/02/2025
Accepted on: 14/05/2025
Available Online: 03/07/2025

Key words:
Anatomical analysis, biochemical analysis, pigeon pea, phytophthora blight, morphological, PR Proteins

ABSTRACT

Pigeon pea (*Cajanus cajan* L), is a perennial crop plant of arid and semiarid regions and its growth has been affected by many biotic stresses and phytophthora blight is one of them. *Phytophthora drechsleri* f. *sp cajani* is a causative agent of phytophthora blight disease in pigeon pea. The morphological, anatomical, and biochemical analysis of two local pigeon pea varieties Shrawani and Shweta for phytophthora infection was assayed in this study. The morphological investigation showed that the infection significantly decreased root length; shoot length, number of leaves, fresh weight, and dry weight by 0.5–2 fold in the Shrawani variety, compared to Shweta. In anatomical analysis, infected Shrawani especially showed signs of cellular damage and mycelial invasion, but the infected Shweta variety showed no symptom of fungal development in another part of the plant except in the roots by the 20th day of infection. The results of the biochemical analysis were related to the morphological and anatomical analysis. These results highlight the strong defensive mechanisms in Shweta, which was characterized by prolonged biochemical responses and enhanced lignification. These findings propose a piece of critical valuable information for screening pigeon pea varieties for resistance toward phytophthora.

1. INTRODUCTION

The pigeon pea *Cajanus cajan* (L.) Mill sp is widely recognized as a species of legume family, that been cultivated in the semi-arid regions of the Indian subcontinent. Since it has a high nutritional value and greater potential to withstand drought, pigeon pea are the most widely grown legume crop and are primarily grown in rainfed fields of tropical and subtropical regions of the world and more than 80 countries cultivating it as a major source of pulses [1]. In some, normally it has been locally recognized as congo pea, no-eye pea, tur dal, arhar, red gramme, thuvaramparruppu, and tomarapayarru in different regions of India. In India, each state has its name for them and uses them in different methods for recipes. Around 5% of the world's pulses belong to pigeon peas. About three-quarters (77.5%) of the world's acreage is used for its cultivation and four-fifths (81.3%) of its total production comes from India alone. Asia is essentially the only region that contributes 2.85 million tonnes of the world's production in acreage (4.16 million ha) [2].

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Nonetheless, pigeon pea crop production in terms of both yield and quality is frequently impacted by a wide range of biotic and abiotic stresses. The pigeon pea crops get infected by a wide range of harmful microbes, including nematodes, insects, bacteria, viruses, and fungi, which is responsible for a potential negative impact on the yield of pigeon pea crops [3]. A serious biotic stressor, the fungal (oomycete) pathogen *Phytophthora drechsleri* f. sp *cajani*, which causes *Phytophthora* blight in pigeon pea plants, is responsible for significant yield losses. It can cause complete plant mortality and complete yield loss, especially during the growing season [4], and the effect gets augmented under extended hot weather and with high humidity. It interspersed rapidly within a week of heavy rainfall in entire crop fields [5]. The severity of the disease can be understood by the phytophthora blight outbreak at the Deccan plateau of India, which had shown complete 100% yield losses due to this disease irrespective of soil types, cultivars, and cropping system in the year 2005 [6]. According to previous studies, the disease significantly reduces productivity not just on the Deccan plateau of India but also in Uttar Pradesh [7]. Many chemical pesticides and fungicides are used to reduce the disease severity to prevent crop loss, but this led to the evolution of chemical-resistant pathogens as well as poor soil fertility. To overcome the problem related to chemical input, alternative biocontrol agents were tested, being eco-friendly; they are

inefficient for more advanced management. So, to sustain the pigeon pea productivity, cultivation of the resistance variety is still the only option, and it requires more pronounced protection measures against this pathogen. Although growing resistant varieties is not only the solution, understanding the nature of resistivity and the mechanism behind that can lead to developing more resistant varieties [8].

Upon pathogen attack the plant's defense mechanisms are triggered by a variety of cellular signals and pathogen penetration triggers the first level of defense mechanism to enable quicker identification of possible invaders [9]. Rapid defense response is necessary to limit pathogen progression in the plant cells, which is achieved by enhanced lignification of the plant's cell wall and the production of numerous vital protective metabolites and phytochemicals such as pathogenesis-related (PR) proteins. Induction of PR proteins such as beta-glucanase and chitinase are involved in the second line of defense [10]. During plant-fungal interactions, an intricate interplay takes place between fungal invasion, colonization methods, infection potential, and plant defense mechanisms, which ultimately results in plants' susceptibility or resistivity toward the pathogen [11]. Critical insight into mechanisms of resistance against certain diseases can be gained by comprehending plant defense mechanisms at different levels. It will be possible to build more potent disease management measures to counter pathogen invasion and increase crop productivity by applying such resistance mechanisms. The present study aimed to understand the plant defense mechanism during fungal pathogen attack under optimal environmental conditions and the effect of phytophthora blight infection was analyzed as the morphological, anatomical, and biochemical changes in the plants against fungal infection.

2. MATERIALS AND METHODS

2.1 Procurement of Pigeon Pea Varieties and Fungal Pathogen

The seeds of pigeon pea varieties were purchased from a local Agro shop at Anand, Gujarat, India, and the fungal pathogen *P. drechsleri* f. sp. *cajani.* isolate was obtained from the Indian Pulse Research Centre Kanpur, Uttar Pradesh, India.

2.2 Effect of *p. drechsleri* f. sp. *cajani* on the Plant Morphology

The experiment used earthen pots of 26.67 cm diameter and 25.4 cm deep, filled with sandy loam soil. Pigeon pea seeds (Shrawani and Shweta) were surface sterilized for 2 minutes using 0.1% mercuric chloride, then washed with sterile distilled water thrice and placed in the pots, with three seeds per pot. A suspension of zoospores (10 ml^{-1} seedling) containing 1.5×10^5 zoospores ml^{-1} was used to infect the 10-day-old seedlings, while sterile distilled water was used as an inoculant for the control plants. Data on morphological traits were gathered for 45 days, starting on the fifth day following inoculation, at intervals of 5 days. (experiment conducted in triplicates). The root length, shoot length, fresh weight, and dry weight were manually done and collected data were subjected to the Univariate analysis of variance, mean and standard deviation recorded, and posthoc tests were performed using the Duncan test.

2.3 Percent Disease Incidence (PDI) in Pigeon Pea

Two pigeon pea varieties from Gujarat were screened for resistance and susceptibility against phytophthora blight disease using four different inoculation methods (mycelial mesh, node, zoospore suspension, and detached leaf method) to determine the most effective mechanism for manual infection and then the zoospore method was used for infection to determine the average PDI. The resistance and susceptibility were determined based on the range of PDI.

2.4 Anatomical Study of Effect of Fungal Pathogen *P. drechsleri* f. sp. *cajani*

The 10-day-old plants (Shrawani and Shweta varieties) were inoculated with 10 ml of 10^5zoospore ml^{-1} suspension of *Phytophthora* f. sp. *Cajani* and leaves, stems, and roots were taken from the 20th day after infection (DAI) in both non-infected and infected pigeon pea plants (Shrawani and Shweta). The anatomical section was prepared by the free-hand method and sections of leaves, stems, and roots were observed under the light microscope attached to a rolled camera (Kodak Easyshare C813 Zoom Digital Camera).

2.5 Estimation of Total Phenolics

Dried leaves powder (20 g) was packed into a Soxhlet apparatus and extracted with 300 ml methanol at 60°C–65°Cfor 3–4 hours. The extract was filtered through Whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure at 40°C. The extract was dried, weighed (2.6 g), and stored at 4°C in storage vials for experimental use. The total phenol and flavonoid content of the extract was determined by the Folin–Ciocalteu method [12]. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per gram dry weight.

2.6 Estimation of Total Flavonoid

The total flavonoid content of the tissue from pigeon pea leaves was measured using the El-Haci *et al.* technique [13]. The plant sample was prepared by homogenizing 1 g of pigeon pea leaves in 10 ml of methanol. The reaction mixture contained 2 ml of distilled water, 3 ml of 5% $NaNO_2$, and 3 ml of 10% aluminium chloride, and incubated at room temperature for 6 minutes, after that 2 ml of 1M NaOH solution was added. After mixing thoroughly, the mixture was allowed to stand for a further 15 minutes and absorbance was recorded at 510 nm using a standard consisting of 0.5 mg^{-1}ml of quercetin mixture and the total flavonoid content was expressed as mg QE^{-1}gram fresh weight.

2.7 Biochemical Analysis of the Effect of *P. drechsleri* f. sp. *cajani*

The 10 days old pigeon pea plant (Shrawani and Shweta varieties) were inoculated with 10 ml of 10^5 zoospore/ml suspension of *Phytophthora* f. sp. *cajani* and used for biochemical analysis. The fresh leaf extract from both infected and control plants were used as a source of enzymes and used for the beta-glucanase and chitinase activity after the fungal infection. The leaf sample was collected at six distinct time points: pre-infection, first, fourth, seventh, ninth, and eleventh DIA.

2.8 Estimation of Beta-glucanase Activity

The beta-glucanase enzyme activity was measured using the laminarin-dinitro salicylic acid approach [14]. The assay mixture (62.5µl of 4% laminarin and 62.5µl of plant extract) was incubated at 40°C for 10 minutes. Then 375 µl of the dinitro salicylic acid reagent was added to stop the reaction and the reaction mixture was placed boiling water bath for 5 minutes. The 0.5 ml of the dark brown reaction mixture was diluted with 4.5 ml of distilled and absorbance at 500 nm was measured. The beta-glucanase enzyme activity is expressed in the µ/mg protein.

2.9 Estimation of Chitinase Activity

The chitinase enzyme activity was measured using the Boller and Mauch technique [15]. The reaction mixture comprised 0.2 ml enzyme, 0.2 ml of 10 mM sodium acetate buffer, and 0.2 ml of 0.05% chitin (Sigma) dissolved in boiling water. The reaction mixture was incubated for an hour at 50°C and the synthesis of sugar N-acetyl glucosamine was assessed using the DMAB technique, followed by 2 minutes centrifugation of the assay mixture at 2,000 rpm [16]. The enzyme activity of chitinase was quantified in the µ/mg protein.

2.10 Statistical Analysis

The data were analyzed statistically following the method of analysis of variance. The statistical analysis was carried out by SPSS 21 statistical software (SPSS Inc.). Mean values were statistically compared by Duncan's multiple range test. It was significant at 0.05% level. The data reported in the graphs were means of 3 replications, and all the treatments were repeated three times. The visual representation data in the form of graphs was made by using GraphPad Prism.

3. RESULTS AND DISCUSSION

The world's continuously growing population requires special attention to overcome the problem of food scarcity in the coming years. Pigeon pea is one of the most important legume crops of the subtropics and tropics, whose productivity is adversely affected by a group of biotic stressors as pathogens, insect-pests, which cause different types of diseases like phytophthora blight, Fusarium wilt, Sterility mosaic disease, and so on. The substantial crop losses caused due to such harmful organisms need to be reduced for the sustainable food supply required for the growing population [17]. Among these phytophthora blight is recognized as a potentially devastating disease for farmers.

3.1. Effect of *P. drechsleri* f. *sp. cajani* on the Plant Morphology

The two pigeon pea varieties Shrawani and Shweta were infected with phytophthora zoospores suspensions at favorable growth conditions of the pathogen to carry out its ability to overcome fungal infection. The pigeon pea varieties Shrawani and Shweta were infected with the fungal pathogen, *P. drechsleri* f. sp. *cajani* showed a notable difference in morphological traits. The data of the experiment indicates that the infected Shrawani had a significant lower root and shoot growth than the non-infected Shrawani and the infected Shweta. There was minor variation in the number of leaves across the groups on the 20th day of infection which may be related to the slow rate of pathogen infection progression toward leaves. The fresh and dry weight data of the experiment also showed that the infected Shrawani's fresh weight was at least half that of the non-infected, which suggests that the infection in Shrawani progresses more quickly than in infected Shweta. This indicates that Shweta had some sort of defense mechanism that halted the infection from further progression. The non-infected varieties Shrawani and Shweta exhibited a continuous increase in all growth parameters during the study period; however, the infection resulted in a significant decline in all growth parameters of the Shrawani variety compared to the infected Shweta variety as shown in Table 1. Piquerez *et al.* [18] reported that the growth and development of resistant variety wheat has been maintained under infection of *Puccinia graminis* f. sp. *tritici*, a causative agent for wheat stem rust [18].

3.2 PDI in Pigeon Pea

Plant disease outbreaks are a common threat to global food security and environmental sustainability, leading to decreased productivity and loss of yield, and climate change exacerbates the risk of infection by influencing the evolution of pathogens and their efficient interactions with host plants as well as favour pathogens spread to new regions responsible for increasing incidence of plant diseases. In this study, the two Gujarat local pigeon pea varieties were analyzed for percent incidence after phytophthora infection, where the Shrawani showed an average of 39.37% PDI, while the Shweta at 15.77%. Based on their average PDI%, Shrawani was moderately susceptible to Phytophthora blight disease and Shweta showed resistant characteristics as shown in Table 2. The infection caused drastic changes in the young plant leaves include rapid yellowing and wilting that is typically accompanied by a soft rot and collapse of the rot and on the basis of the morphological data of phytophthora blight disease using four different inoculation methods using the zoospore-treated and non-treated pigeon pea seeds showed that the Shrawani variety was moderately susceptible to *Phytophthora* blight disease and Shweta showed resistance. Van Dijk *et al.* [19] also reported that the fungal-infected plants are expected to have reduced resources for growth and reproduction as defence is costly and pathogens withdraw nutrients from the host.

3.3 Anatomical Study of Effect of *P. drechsleri* f. sp. *cajani*

Some phenolic compounds, like lignin, are essential to the formation and strengthening of plant cell walls; lignin plays a crucial role in

Table 1. Percent disease incidence of the pigeon pea varieties.

Pigeon pea varieties	Percent disease incidence (%)	Characteristic
Shrawani	39.36	Moderately susceptible
Shweta	15.77	Moderately resistant

Table 2. Effect of *phytophthora drechsleri* f. sp. *cajani* on plant growth parameter in 1 month old pigeon pea plants 20 days after infection.

Pigeon pea varieties	Treatment	Root length (cm)	Shoot length (cm)	Number of leaves	Plant spread (cm)	Fresh weight (g)	Dry weight (g)
SHRAWANI	Non-infected	5.82 ± 0.15^{b}	23.88 ± 0.08^{b}	9.67 ± 0.58^{b}	40.12 ± 0.22^{b}	2.15 ± 0.02^{b}	0.61 ± 0.02^{b}
	Infected	4.81 ± 0.07^{a}	18.4 ± 0.32^{a}	6.33 ± 0.58^{a}	23.14 ± 0.11^{a}	1.18 ± 0.04^{a}	0.24 ± 0.03^{a}
SHWETA	Non-infected	5.76 ± 0.11^{b}	23.54 ± 0.3^{b}	11.00 ± 1.00^{b}	$46,24 \pm 0.06^{b}$	2.16 ± 0.04b	0.93 ± 0.02^{d}
	Infected	4.78 ± 0.18^{a}	21.8 ± 0.46^{b}	9.67 ± 0.58^{b}	28.4 ± 0.14^{a}	1.12 ± 0.07^{a}	0.8 ± 0.02^{c}
	Mean square	0.989	18.880	11.889	35.212	1.002	0.275
	F (df = 3,8)	54.458	185.569	23.778	121.11	500.911	734.304
	Significant	0.000	0.000	0.000	0.000	0.000	0.000

Based on Duncan's post-hoc test, different letters (a, b, c, and d) within each column indicate significant differences between groups at $p < 0.05$.

lignification, which makes cell walls more rigid and less permeable to pathogens [20]. Apart from this, phenolic compounds can also serve as signaling molecules for the activation of the defense mechanism. Plant upon pathogenic microbial interaction, activates diverse mechanisms starting with the activation of primary barriers like cell wall reinforcement by lignin, suberin, and callose deposition in the cell as well as the production of antimicrobial peptides, enzymes, and secondary metabolites. In the present study, transverse sections of both Shrawani and Shweta varieties were done on the 20th DAI. The observation revealed that infected Shrawani showed signs of pathogen mycelial growth with the typical symptoms of root structure and remarkable destruction of epidermal cells. The fungal pathogens invasion in the xylem and phloem vessels had also been recorded. At the same, the infected Shweta exhibited a small amount of pathogen invasion on 20th DAI, with well-organized secondary xylem and phloem vessels. Additionally, enhanced lignification of the cell walls is visualized as dark purple vessels in the root section of Shweta (Fig. 1). Lee *et al.* [21] also reported that lignin plays an essential regulatory role in plant immunity and lignin deposition readily occurs during plant–pathogen interaction to develop a barrier for further fungal invasion in resistance plant variety.

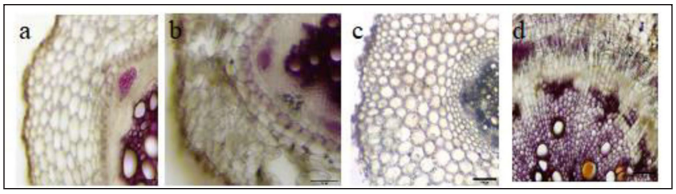

Figure 1. Transverse section of both infected and non-infected pigeon pea plant roots on the 20th days after Infection (DAI), 10x and 100µm scale bar. (a non-infected Shrawani at 20th DAI, b- Infected Shrawani at 20th DAI, c- non-infected Shweta at 20th DAI, d- infected Shweta at 20th DAI.

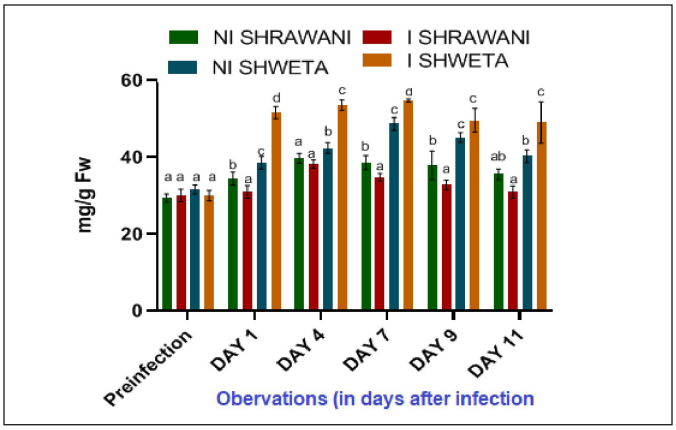

Figure 2. Estimation of the total phenol content of pigeon pea varieties on pre-infection and post-infection days. Values represent mean ± S.D. (NI- control, I- Infected) Based on Duncan's post-hoc test, different letters (a, b, c, and d) within graph indicate significant differences between groups at $p < 0.05$.

3.4 Estimation of Total Phenolic

Simple phenols, phenolic acids, flavonoids, and lignin are among the diverse group of secondary metabolites known as phenolic compounds, and they play a vital role in plant defense against pathogens and pests through a variety of mechanisms. Some phenolic compounds, like stilbenes and flavonoids, act as phytoalexins [22]. The production of phenolic compounds by the plant under stress takes place by the shikimate–phenylpropanoid pathway, which stimulates the production of a biochemically diverse class of compounds having varied diverse functions in plant protection. The result of the total phenolic content of the infected Shrawani was significantly lower than Shweta and maximum phenolic in infected Shrawani was recorded on the 4th DAI (38.31 mg^{-1}gram fresh weight), which get declined from the 7th DAI to34.82 mg^{-1}gram fresh weight. Compared to infected Shrawani, infected Shweta exhibited a striking rise in phenolic from 30.06 mg^{-1}gram fresh weight and peaking at 54.84 mg^{-1}gram fresh weight at the 7th DAI, which indicates that, pigeon pea variety Shweta was able to increase the production of the phenolic compound upon fungal interaction to prepare the plant sufficiently to fight against pathogen infection (Fig. 2). Dehghanian *et al.* [23] also reported enhanced accumulation of phenolic in plants like cucumber, soybean, tobacco, and cotton, which is recognised by plant pattern diagnosis receptors leading to PAMP-triggered immunity in the plant, which halted the disease progression before the pathogen gains full control of the plant [23].

3.5 Estimation of Total Flavonoid

Flavonoids are essential molecules affecting plant growth and development, as well as acting on plant defense mechanisms against biotic and abiotic factors. Plants upon interaction with pathogen, induce the production of flavonoid compounds having antimicrobial activity, which get accumulation in the plant infected and surrounding tissues to repress the infection and its spreading [24]. In the present study, infected Shrawani showed a drastic reduction in the total flavonoid content upon the fungal infection and not able to overcome fungal infection. Shrawani showed a slight increase in the total flavonoid content upon fungal infection and reach peak at the 4th DAI with only 6.76 mg QE/gram fresh weight, then it drastically get reduced upto 1.30 mg QE/g fresh weight by 11th DAI. While, infected Shweta showed a prominent increase from the pre-infection to a maximum of 24.60 mg^{-1}gram fresh weight on 7th DAI, which was nearly 2folds higher than non-infected Shrawani (Fig. 3), and able to maintain its growth normal under fungal infection and able to over the adverse effect of fungus. Long *et al.* [25] also reported that, accumulation of flavonoid in cotton is responsible for provide better disease resistance potential. Wang *et al.* [24] also reported that transgenic expression of flavanone 3-hydroxylase redirects flavonoid biosynthesis and alleviates anthracnose susceptibility in sorghum and the reduced accumulation of flavonoids can enhance the susceptibility [24].

3.6 Estimation of Beta-glucanase Activity

After primary barriers are assaulted by the phytopathogen, the plant activates a cascade of defense responses like induction of protective proteins for its protection against phytopathogens known as PR proteins. The β-1,3- glucanase hydrolyses β-1,3-linked glucans, the major structural component of fungal cell walls and are responsible for the induction of plant defence mechanisms against fungal infection [26]. The production of PR proteins is common feature of a plant to fight against pathogens and in this study, the production of protective beta-glucanase and chitinase enzymes was considerably high in plants after fungal infection. The beta-glucanase activity increased gradually from 1.38 µ/mg to 1.68 µ/mg upon fungal infection and then gradually decreased to 0.67 µ/mg at the 11^{thc} DAI in pigeon pea variety Shrawani. However, infected Shweta showed a remarkable rise in beta-glucanase activity during the initial stage of the infection of 1.40 µ/mg to 9.23 µ/mg up to the 7th DAI, which was nearly 2.5

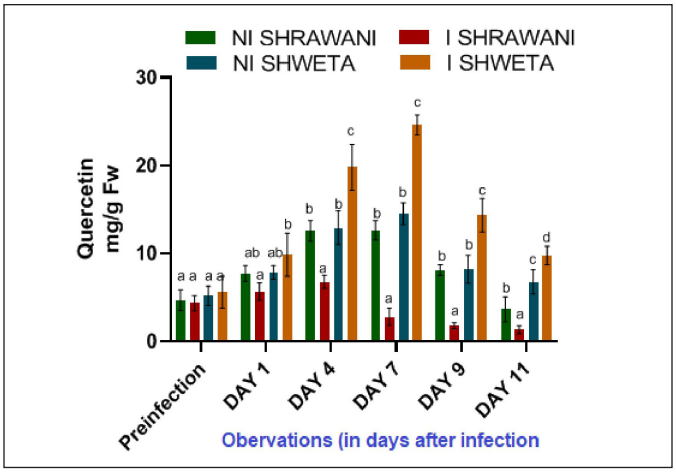

Figure 3. Estimation of the flavanoids content of pigeon pea varieties on pre-infection and post-infection days. Values represent mean ± S.D. (NI- control, I- Infected) Based on Duncan's post-hoc test, different letters (a, b, c, and d) within graph indicate significant differences between groups at $p < 0.05$.

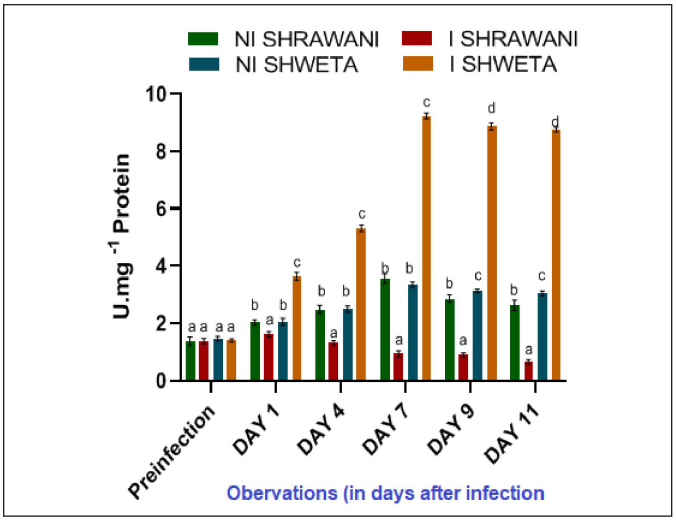

Figure 4. Beta-glucanase activity of pigeon pea varieties on pre-infection and post-infection days. Values represent mean ± S.D. (NI control, I- Infected) Based on Duncan's post-hoc test, different letters (a, b, c, and d) within graph indicate significant differences between groups at $p < 0.05$.

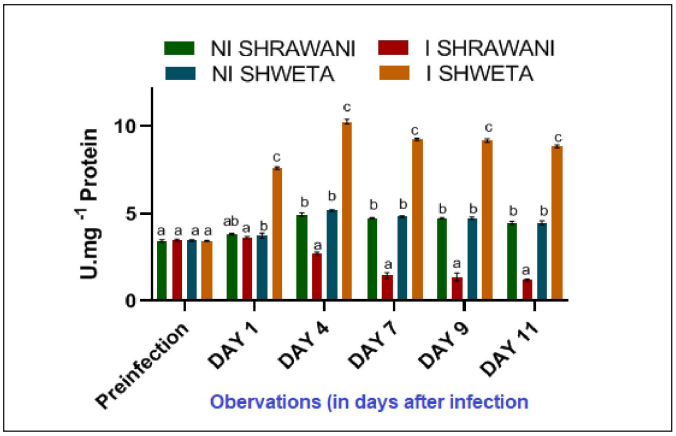

Figure 5. Chitinase activity of pigeon pea varieties on pre-infection and post-infection days. Values represent mean ± S.D. (NI- control, I- Infected) Based on Duncan's post-hoc test, different letters (a, b, c, and d) within graph indicate significant differences between groups at $p < 0.05$.

times greater than Shrawani (Fig. 4). Wang *et al.* [27] reported that, the *Magnaporthe oryzae* fungus interaction with rice induce induction of β-1,3-glucanase enzyme activity, which help rice to overcome the fungal infection by direct antifungal effect of the β-1,3-glucanase dependent hydrolysis of *Magnaporthe oryzae* [27]. Plant β-1,3-glucanase are reported to activate unconventional plant immune responses by release β-1,3-glucan oligosaccharides as a catabolic product hyphal cell wall of degradation of attacking fungi, which act as elicitors. These elicitors recognised by plant defence systems and trigger the signalling cascades for the activation of a wide range of localized and systemic defence responses in plant to fight against fungus in subsequent attacks [28].

3.7 Estimation of Chitinase Activity

Chitinases belong to the class of hydrolytic enzymes that hydrolyze the N-acetylglucosamine polymer of chitin, having the *potential to inhibit or degrade* chitin-containing microorganisms like fungi and insects [29]. The enzyme may be expressed constitutively at low level in plants, but are dramatically enhanced by numerous abiotic agents and biotic factors. The result of the present study showed that infected Shrawani had a continuous reduction in the chitinase activity from pre-infection 3.47 µ/mg protein to 1.87 µ/mg proteins at 11th DAI. However, infected Shweta showed a noteworthy increase in chitinase enzyme activity from the pre-infection 3.42 µ/mg protein to a maximum of 10.26 µ/mg protein at the 4th DAI, which is 2 times higher than Shrawani. Both the non-infected Shrawani and Shweta showed similar patterns of chitinase activity, an initial increase from pre-infection to the maximum at 4th DAI, followed by a remarkable reduction in the chitinase activity was observed at 9th DAI and 11th DAI, respectively (Fig. 5). Additionally, these enzymes after hydrolysis of fungal cell wall release by-product act as elicitor molecules, which is recognised by plant cell receptors to initiate the downstream signal transduction process for the accumulation of such enzymes resulting in the systemic acquired resistance [30]. Zhou *et al.* [31] also reported that, in apple plant induction of chitinase enzyme activity is responsible for the degradation of chitin component of fungal cell wall, to provide resistance to apple against fungal infection, not only that the chitinase enzyme change the cell polarity of pathogenic fungi by binding at the tip of fungal hyphae [31].

4. CONCLUSION

The present study involves anatomical, morphological, and biochemical assessment of phytophthora blight-infected pigeon pea plant varieties Shrawani and Shweta on 20th DAI the study showed that the phytophthora blight infection had a severe adverse impact on the growth of the two pigeon pea varieties. Infected Shrawani showed rapid and massive cell damage, cell wall disturbance and vascular bundle disruption, which hindered the morphological growth. On the contrary, infected Shweta demonstrated well-organized cell structures and improved lignification giving strength to the cell wall; continuous and steady morphological traits throughout the experiment along enhanced biochemical parameters resulted in enhancing the plant's overall defence mechanism and protecting it against the fungal pathogen. So, the study showed that the pigeon pea variety Shweta is a resistant variety to phytophthora blight disease and these parameters can be used for the selection of breed for the breeding program for the development of phytophthora resistant variety.

5. ACKNOWLEDGMENT

The authors are immensely grateful to the Gujarat government for providing them the financial assistance as the Shodh fellowship for conducting this research work.

6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

10. PUBLISHER'S NOTE

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The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:
Yadav KA, Naik SSJ, Chauhan V, Yadav N, Jha Y. Effect of *Phytophthora drechsleri* f. sp *cajani* infection on local pigeon pea variety of Gujarat. J Appl Biol Biotech. 2025;13(Suppl 1):62–68. DOI: 10.7324/JABB.2025.214212.