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Morpho-Anatomical and Biochemical Defense Responses of Pigeon Pea Varieties to Phytophthora Blight

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ABSTRACT: Phytophthora blight is a devastating disease of pigeon pea (*Cajanus cajan*) that severely impacts plant growth and productivity. This study investigates the morphological, anatomical, and biochemical responses of a susceptible variety (ICPL 11260) and a resistant variety (IPAC-02) following infection by Phytophthora. Morphological analyses showed that infection caused a drastic reduction in root length, shoot length, leaf number, fresh weight, and dry weight in the susceptible ICPL 11260 variety, with reductions ranging from 0.5- to 2-fold compared to non-infected controls. Anatomical observations revealed pronounced cellular damage and mycelial invasion in infected ICPL 11260 plants by 30 days after infection, whereas infected IPAC-02 plants exhibited no fungal colonization. Biochemical analyses further demonstrated that the resistant IPAC-02 variety accumulated higher levels of total soluble sugars, proteins, phenols, and flavonoids, along with increased activities of defense-related enzymes (chitinase and β -1,3-glucanase), compared with the susceptible ICPL 11260. Under *P. cajani* stress, IPAC-02 maintained significantly elevated osmolyte concentrations (total sugars 153.7 mg g⁻¹ FW; proteins 25.4 mg g⁻¹ FW), secondary metabolites (phenols 51.7 mg g⁻¹ FW; flavonoids 33.1 mg g⁻¹ FW), and PR-enzyme activities (chitinase 11.4 U mg⁻¹ protein; β -1,3-glucanase 9.1 U mg⁻¹ protein). These responses support a lignification-mediated defense mechanism in IPAC-02 and highlight its potential value for breeding Phytophthora-resistant pigeon pea cultivars.

KEYWORDS: Defense-related enzymes; flavonoids; phenolics; protein; soluble sugars

1 Introduction

Pigeon pea (*Cajanus cajan* L.) is a multipurpose perennial legume widely cultivated across tropical and subtropical regions. It is a major source of plant-based protein, dietary fiber, essential vitamins, and beneficial phytochemicals for millions of people, particularly in vegetarian and low-income populations [1]. Beyond its nutritional value, pigeon pea cultivation plays a crucial role in supporting the livelihoods of subsistence farmers in South Asia, East Africa, and parts of South America [2]. The crop is also used in various value-added products, including flour, noodles, biscuits, and bread [3]. Globally, approximately 4.49 million tons of pigeon peas are produced annually, with India accounting for nearly 63% of total production and 72% of the global cultivation area [4].

Despite its importance, pigeon pea productivity remains low due to biotic stresses, particularly fungal and oomycete pathogens such as dry root rot, Fusarium wilt, sterility mosaic disease, powdery mildew, and Phytophthora blight [5,6]. Among these, Phytophthora blight, caused by *Phytophthora cajani*, is one of the most destructive diseases, capable of causing severe plant mortality and significant yield losses [7]. The pathogen, predominantly soil- and water-borne, infects pigeon pea at any growth stage and induces symptoms such as stem blight, stem canker, leaf blight, and root rot [8]. Disease outbreaks are particularly common under conditions of prolonged humidity, warm temperatures, and excessive rainfall, making it a recurring threat in major pigeon pea-growing regions [7–10]. The response of a genotype to biotic stress is largely determined by its physiological and biochemical attributes, which play a crucial role in conferring either resistance or susceptibility. Plants are equipped with a wide range of defense mechanisms that restrict the growth and development of pathogenic organisms. Key biomolecules associated with disease resistance include polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), peroxidase (POD), phenolic compounds, soluble carbohydrates, and ascorbic acid. Investigating the biochemical basis of resistance enhances our understanding of host–pathogen interactions and provides valuable insights for improved disease management strategies [11].

Plants respond to pathogen invasion through a complex and multilayered defense system involving morphological, anatomical, and biochemical mechanisms. Structural defenses, such as cell wall thickening, lignification, and modifications in xylem and phloem tissues, act as early barriers that restrict pathogen penetration and colonization [12]. At the biochemical level, pathogen attack triggers the accumulation of reactive oxygen species, osmolytes, secondary metabolites (phenols, flavonoids), and pathogenesis-related (PR) proteins, including chitinases and β -1,3-glucanases, which collectively contribute to induced resistance [12,13]. The magnitude of these responses varies among genotypes and is often associated with the degree of disease resistance they exhibit [6,9,13].

Although several studies have examined individual biochemical or physiological responses to Phytophthora blight, an integrated understanding of how morphological damage, anatomical alterations, and biochemical defense activation collectively determine resistance in pigeon pea remains limited. A comprehensive comparative analysis of resistant and susceptible genotypes is essential for elucidating defense pathways and identifying traits that contribute to durable resistance. Therefore, the present study investigates the morpho-anatomical and biochemical defense responses of a resistant pigeon pea variety (IPAC-02) and a susceptible variety (ICPL 11260) following infection with *Phytophthora cajani*. By assessing growth parameters, tissue-level anatomical changes, osmolyte accumulation, secondary metabolite production, and PR-enzyme activities across different days post-infection, this study provides a holistic understanding of the defense mechanisms governing varietal resistance. The findings contribute valuable insights for breeding programs aimed at developing Phytophthora-resistant pigeon pea cultivars.

2 Materials and Methods

2.1 Plant Materials and Fungal Pathogens

Seeds of the pigeon pea varieties, including the Phytophthora blight–susceptible ICPL 11260 and the Phytophthora blight–resistant IPAC-02, along with the *P. cajani* isolate, were obtained from the Indian Pulse Research Centre, Kanpur, Uttar Pradesh.

2.2 Zoospore Production and Culture Conditions

Zoospore production was carried out by culturing *P. cajani* on V8 juice agar medium under controlled laboratory conditions. Actively growing mycelial discs were transferred to sterile distilled water and

incubated under continuous light at 24–26°C to stimulate sporangial formation. Subsequent chilling at 4–8°C for 30–60 min, followed by incubation at room temperature, induced zoospore release. The resulting zoospore suspension was filtered to remove mycelial debris and adjusted to the required concentration using a hemocytometer for further experimental use. Zoospore viability was checked before inoculation by spreading an aliquot onto 1.5% water agar and assessing germination after 2–3 h; only suspensions with >90% germinating zoospores were used.

2.3 Experimental Design

Earthen pots with a diameter of 10.5 inches and a depth of 10 inches, filled with sandy loam soil, were used for the experiment. Pigeon pea seeds (ICPL 11260 and IPAC-02) were surface-sterilized with 0.1% mercuric chloride for 2 min, followed by thorough washing with sterile distilled water, and then planted in the pots at a rate of three seeds per pot. The experiment was conducted using a completely randomized design (CRD) consisting of 100 pots in total. Two pigeon pea (*C. cajan*) varieties were evaluated, each represented by 50 pots. For each variety, two treatments (inoculated and non-inoculated control) were applied, with 25 pots per treatment. Each pot contained uniform, healthy seedlings and was considered an experimental unit, as all plants within a pot were subjected to the same treatment and maintained under identical growth conditions. Five-day-old seedlings were inoculated with 10 mL per seedling of a zoospore suspension containing 1.5×10^5 zoospores/mL, while control plants received sterile distilled water.

2.4 Growth Conditions

All pot experiments were conducted in a controlled growth chamber. The temperature was maintained at $25 \pm 2^\circ\text{C}$ during the light period and $20 \pm 2^\circ\text{C}$ during the dark period, with a relative humidity of 60–70%. A 16/8 h light/dark photoperiod was provided, and photosynthetically active radiation at canopy height was maintained at $250\text{--}300 \mu\text{mol m}^{-2} \text{s}^{-1}$ using cool white fluorescent lamps. Pots were randomly rearranged every 3–4 days to minimize positional effects within the chamber.

2.5 Soil Collection and Characterization

The experiment was conducted using surface soil (0–15 cm) collected from the experimental farm. The soil was air-dried, gently crushed, and passed through a 2 mm sieve before use. Soil texture was determined using the hydrometer method and confirmed as sandy loam. Soil pH and EC were measured in a 1:2.5 (w/v) soil-to-water suspension using a digital pH and conductivity meter, and organic matter content was determined using the Walkley–Black method. The main physico-chemical properties of the soil used in the pot experiment are presented in Table 1. To maintain the natural microbial community relevant for disease development, the soil was used in a non-sterilized form after sieving and homogenization.

Table 1: Physico-chemical properties of the soil used in the pot experiment.

Parameter	Value	Method
Texture	Sandy loam	Hydrometer method
Sand (%)	62.4	
Silt (%)	23.5	
Clay (%)	14.1	
pH (1:2.5 soil:water)	7.2	Digital pH meter
Electrical conductivity (EC)	0.72 dS m^{-1}	Conductivity meter (1:2.5 soil:water)
Organic matter (%)	0.65	Walkley–Black method
Available N (kg ha^{-1})	145	Kjeldahl
Available P (kg ha^{-1})	18.2	Olsen (NaHCO_3 extraction)
Available K (kg ha^{-1})	165	Flame photometer

2.6 Data Collection and Morphological Parameters

The entire experiment was replicated three times under identical environmental conditions. Data on morphological traits were collected at five-day intervals initially, and then at ten-day intervals, starting from the 5th day after inoculation and continuing for 35 days. Shoot length was measured using a ruler from the collar region to the tip, and root length was similarly measured from the collar region to the tip of the primary root. The number of leaves was counted manually. Whole plants were carefully uprooted and weighed using an electronic balance to record fresh weight. The uprooted plants were then dried in an oven for 24 h and weighed again to determine their dry weight.

2.7 Disease Assessment and Symptom Scoring

Disease development following *P. cajani* inoculation was evaluated using a visual 0–5 disease severity scale using the formula:

Disease index = Total grade \times 100/No. of sheaths observed \times maximum grade.

Disease ratings were conducted at 7, 14, 21, 28, and 35 days post-inoculation (dpi) across all genotypes and treatments. Mean disease severity values were computed per pot and used as biological replicates for statistical analysis.

2.8 Pathogen Re-Isolation and Molecular Confirmation

To fulfill Koch's postulates *P. cajani* was re-isolated from symptomatic root and stem tissues collected at 21 and 35 dpi. Tissue segments were surface-sterilized (70% ethanol followed by 1% sodium hypochlorite for 1 min each), rinsed in sterile water and plated on selective V8 agar amended with antibiotics. Emerging colonies were identified based on colony morphology, sporangial characteristics, and growth patterns consistent with original inoculum cultures.

2.9 Anatomical Studies

The 15-day-old Phytophthora blight-susceptible (ICPL 11260) and resistant (IPAC 02) pigeon pea varieties were inoculated with 10 mL of a 10^{-5} zoospore/mL suspension of *P. cajani* for anatomical studies. Leaves, stems, and roots from both control and infected plants of each variety were examined. Three observation time points—pre-infection, 15 days after infection, and 30 days after infection—were used, and sections were prepared using the freehand method. All sections were observed under a light microscope equipped with a digital camera.

2.10 Biochemical Analysis

The 15-day-old phytophthora blight susceptible (ICPL 11260) and resistant (IPAC 02) plant varieties were inoculated with 10 mL of a 10^5 zoospore/mL suspension of *P. cajani* for biochemical analysis. Fresh leaf samples of both control and infected plants are used as samples for various biochemical analyses. Samples were taken at six different time points: pre-infection and the 10th, 15th, 20th, 25th, 30th, and 35th days after infection (DAI) for assessing total sugar, total protein, total phenol, total flavonoid, β -glucanase, and chitinase activity concerning fungal infection.

2.11 Determination of Total Soluble Sugar

The phenol-sulphuric acid method, with minor changes, was used to assess the total soluble sugars in the pigeon pea leaves [14]. One gram of pigeon pea leaf tissue was homogenized in 80% ethanol. The

material was refluxed for 15 min at 70°C in a water bath and then centrifuged for 10 min at 6000× g. Two further extractions of the pellet using 80% ethanol were performed, and the supernatants were combined. To estimate the total soluble sugar, a sample of the supernatant was used. Each treatment sample received 0.1 mL of supernatant, 0.9 mL of distilled water, 80 µL of 80% phenol, and 1 mL of concentrated H₂SO₄ in the reaction mixture, which was then thoroughly shaken. At 490 nm, the absorbance was measured using a 1 mg/mL glucose solution as the reference. The amount of soluble sugar in total was stated as mg/g FW.

2.12 Determination of Total Protein

The technique outlined by Lowry et al. [15] was used to estimate total protein. Cold 0.1 M phosphate buffer at pH 7.2 was used to homogenize one gram of pigeon pea leaf tissue. The leaf extract was centrifuged at 10,000× g for 4°C, and the supernatant was taken as a sample to measure the amount of protein. The reaction mixture comprised 5 mL of alkaline copper sulfate solution, 0.9 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and 0.1 mL of supernatant. It was then incubated at room temperature in the dark for 30 min before the absorbance at 660 nm was measured. As a reference, 0.2 mg/mL of bovine serum albumin was used. The total protein content was computed and expressed as mg/g FW.

2.13 Determination of Total Phenol

Khatiwora et al. [16] developed a method to determine the total phenol content of pigeon pea seedlings. In a mortar and pestle, one gram of pigeon pea leaves was homogenized with 10 mL of 80% ethanol. After centrifuging the homogenate for 15 min at 10,000× g, the supernatant was left to evaporate in a water bath. After that, a predetermined amount of hot, distilled water was used to dissolve the residue. 1 mL of an aliquot was added to each test tube, followed by 0.5 mL of Folin-Ciocalteu reagent and, 3 min later, 2 mL of 20% sodium carbonate solution. The mixture's ultimate volume is 5 mL in each tube after adding distilled water. At 660 nm, the absorbance was measured. To plot the standard curve, a concentration of 0.1 mg/mL of catechol was used as the standard. The unit of measurement for total phenol content was mg catechol/g fresh weight of material.

2.14 Determination of Total Flavonoid

The El-Haci et al. [17] method was used to determine the total flavonoid content of the tissue from pigeon pea leaves. One gram of pigeon pea leaves was homogenized with 10 mL of methanol. The reaction mixture consists of 0.2 mL of supernatant with 2 mL of distilled water, 0.3 mL of 5% NaNO₂, and 0.3 mL of 10% aluminum chloride (AlCl₃). It is then allowed to sit at room temperature for 6 min before 2 mL of a 1M NaOH solution is added. Once the mixture was well combined, it was left to stand for an additional 15 min. The mixture was then tested for absorbance at 510 nm with a standard consisting of 0.5 mg/mL of quercetin solution. The overall flavonoid content was expressed as mg quercetin/g fresh weight.

2.15 Determination of β -Glucanase

One gram of leaves was extracted using a pre-chilled mortar and pestle with 1% (w/v) soluble polyvinylpyrrolidone (PVP), 0.2% (w/v) sodium sulfate, and 10% sodium chloride in 10 mL of 100 mM sodium acetate buffer, pH 4.8. After 15 min at 10,000 rpm, the homogenate was centrifuged [18]. The laminarin-dinitro salicylic acid technique was used to assess the beta-glucanase enzyme activity. For 10 min, the assay mixture, which included 62.5 µL of 4% laminarin and 62.5 µL of plant extract, was incubated at 40°C. The reaction was stopped by adding 375 µL of the dinitrosalicylic acid reagent, which was then boiled for 5 min in a boiling

water bath. 0.5 mL of the dark brown solution was diluted with distilled water using 4.5 mL. The absorbance was determined at 500 nm using a UV-Vis spectrophotometer (PerkinElmer Lambda 365).

Reducing sugars released were quantified against a standard curve prepared with glucose ($0\text{--}200\text{ }\mu\text{g mL}^{-1}$; $R^2 \geq 0.99$), and results were expressed as μmol glucose equivalents released. One unit (1 U) of β -1,3-glucanase activity was defined as the amount of enzyme that released 1 μmol of glucose equivalents per minute under the assay conditions, and activity was normalized to protein content and expressed as U mg^{-1} protein. Preliminary assays were conducted to ensure that product formation was linear with respect to incubation time (15–45 min) and protein concentration in the extract, and final reactions were run within this linear range.

2.16 Determination of Chitinase

The extraction was prepared the same as the beta-glucanase assay. The Boller and Mauch [19] method was used to measure the chitinase enzyme activity. The reaction mixture consists of 0.2 mL of the 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 then incubated at 50°C for 1 h. After centrifuging the assay mixture for 2 min at 2000 rpm, p-dimethylaminobenzaldehyde (DMAB) method was used to evaluate the production of the sugar N-acetylglucosamine [20]. Chitinase enzyme activity was measured in U mg^{-1} protein.

Released N-acetyl-D-glucosamine (NAG) was quantified using a standard curve of NAG ($0\text{--}200\text{ }\mu\text{g mL}^{-1}$; $R^2 \geq 0.99$), and activity was expressed as μmol NAG equivalents released. One unit (1 U) of chitinase activity was defined as the amount of enzyme that released 1 μmol of NAG equivalents per minute under the assay conditions, and values were normalized to protein content and reported as U mg^{-1} protein. As with β -1,3-glucanase, preliminary tests confirmed that product formation was linear over the chosen incubation period and protein concentration range, and all final measurements were performed within this linear range.

2.17 Protein Source for Enzyme Normalization and Standard Curves

The protein concentration used to normalize PR-enzyme activities was measured from the same crude extract that was used for enzyme assays. The standards, concentration ranges, and linearity (R^2) for all biochemical assays are as follows:

Soluble sugars: glucose standard (e.g., $0\text{--}100\text{ }\mu\text{g mL}^{-1}$, $R^2 \geq 0.99$)

Total proteins: BSA standard (e.g., $0\text{--}200\text{ }\mu\text{g mL}^{-1}$, $R^2 \geq 0.99$)

Total phenols: catechol standard (e.g., $0\text{--}200\text{ }\mu\text{g mL}^{-1}$, $R^2 \geq 0.99$)

Total flavonoids: quercetin standard (e.g., $0\text{--}100\text{ }\mu\text{g mL}^{-1}$, $R^2 \geq 0.99$)

Extraction blanks (reagent controls without tissue extract) were included for each assay and subtracted from sample absorbance. Each biological replicate was measured with three technical replicates, and mean values were used for analysis.

2.18 Statistical Analysis

Statistical analysis was performed using SPSS software. Data were expressed as mean \pm standard deviation. Initially, a one-way ANOVA was applied with three replicates per treatment, followed by Duncan's multiple range test to compare means, and differences were considered significant at $p \leq 0.05$.

3 Results

3.1 Effect of Infection of *P. cajani* on Morphological Parameters of Pigeon Pea Varieties

A study of morphological parameters in pigeon pea varieties infected with *P. cajani* revealed significant differences between infected and non-infected plants, as well as between susceptible and resistant varieties. As shown in Table 2, the root and shoot lengths of the susceptible variety ICPL 11260 were markedly affected by the pathogen. Across all time points, infected ICPL 11260 consistently exhibited the shortest roots and shoots lengths and the lowest number of leaves compared to non-infected ICPL 11260 and both infected and non-infected IPAC-02. In contrast, IPAC-02 maintained greater root and shoot lengths under both infected and non-infected conditions at most point, suggesting a variety-specific resilience to phytophthora blight. The pattern of leaf development mirrored that of root and shoot growth, with infected ICPL 11260 showing the fewest leaves throughout the experimental period, while non-infected IPAC-02 reached a maximum of 25 leaves on the 45th day and generally maintained the highest leaf numbers across most time points.

The data in Table 2 indicate that infected ICPL 11260 consistently exhibited the lowest fresh weight, approximately half that of non-infected ICPL 11260, from the onset of infection throughout the experiment, reflecting a more progressive impact of *P. cajani* on this susceptible variety compared to infected IPAC-02. Both non-infected varieties showed steady and continuous growth during the experimental period. Notably, infected IPAC-02 displayed a significant increase in dry weight (0.82 g) on the 20th day compared to non-infected IPAC-02 and both ICPL 11260 treatments. This continuous increase in dry weight in infected IPAC-02 suggests the activation of defense mechanisms that protect it against pathogen invasion. In contrast, infected ICPL 11260 consistently recorded the lowest dry weight, approximately 1.5–2 times lower than both non-infected ICPL 11260 and IPAC-02, as well as infected IPAC-02, throughout the experimental period.

The higher dry weight observed in infected IPAC-02 may be attributed to the formation of secondary structures, lignification of cell walls, and synthesis of defense-related metabolites. In contrast, the higher fresh weight in non-infected IPAC-02 reflects efficient water uptake, which may be compromised in infected plants. Fungal invasion likely triggers defense responses that alter normal plant physiological activity to protect against pathogen attack. Both non-infected ICPL 11260 and IPAC-02 maintained continuous and steady growth throughout the experimental period. Overall, the data indicates that *P. cajani* infection caused a significant decline in all growth traits, particularly in the susceptible ICPL 11260 variety.

3.2 Two Way Anova of Morphological Analysis

Assumptions of ANOVA were evaluated prior to analysis. Normality of residuals and homogeneity of variances were assessed using the Shapiro–Wilk and Levene’s tests, respectively. For morphological parameters, these tests indicated deviations from normality and homoscedasticity ($p < 0.05$). However, given the balanced experimental design with equal replication across treatments and time points, ANOVA is considered robust to moderate violations of these assumptions and was therefore applied.

Morphological traits were significantly influenced by variety, treatment, and days after infection (DAI). For root length, three-way ANOVA revealed significant main effects of variety ($F_{1,48} = 103.73$, $p < 0.001$, $\eta^2 = 0.684$), treatment ($F_{1,48} = 67.43$, $p < 0.001$, $\eta^2 = 0.584$), and DAI ($F_{5,48} = 382.62$, $p < 0.001$, $\eta^2 = 0.976$), with a significant variety \times treatment interaction ($F_{1,48} = 11.74$, $p = 0.001$, $\eta^2 = 0.196$). Interactions involving DAI were also significant (variety \times DAI: $F_{5,48} = 6.54$, $p < 0.001$; treatment \times DAI: $F_{5,48} = 5.62$, $p < 0.001$), whereas the three-way interaction was not significant ($F_{5,48} = 2.40$, $p = 0.051$). The residual mean square error (MSE) for root length was 0.108.

Table 2: Effect of *P. cajani* infestation on roots, shoot lengths, and number of leaves of pigeon pea varieties.

Pigeon Pea Varieties	Treatment	Days after Infection (DAI)					
		Root Length (cm)					
		10	15	20	25	30	35
ICPL 11260	Non-Infected	3.88 ± 0.06 ^c	4.93 ± 0.07 ^c	5.71 ± 0.08 ^c	7.14 ± 0.11 ^a	7.76 ± 0.12 ^b	9.29 ± 0.15 ^c
	Infected	3.37 ± 0.22 ^d	4.42 ± 0.22 ^d	5.16 ± 0.26 ^d	6.41 ± 0.58 ^c	6.82 ± 0.46 ^c	7.13 ± 0.25 ^d
IPAC-02	Non-Infected	4.50 ± 0.25 ^a	5.63 ± 0.28 ^a	6.46 ± 0.58 ^a	7.17 ± 0.16 ^a	8.53 ± 0.14 ^a	10.2 ± 0.22 ^a
	Infected	4.11 ± 0.1 ^b	5.12 ± 0.13 ^b	6.26 ± 0.10 ^b	6.74 ± 0.52 ^b	7.87 ± 0.60 ^b	9.53 ± 0.43 ^b
Shoot length (cm)							
ICPL 11260	Non-Infected	18.74 ± 0.4 ^b	21.3 ± 0.20 ^b	22.87 ± 0.2 ^b	25.69 ± 0.1 ^b	32.00 ± 0.4 ^b	41.61 ± 0.3 ^{ab}
	Infected	14.00 ± 0.6 ^d	16.2 ± 0.18 ^c	18.05 ± 0.2 ^c	19.61 ± 0.3 ^d	20.24 ± 0.2 ^c	21.46 ± 0.2 ^c
IPAC-02	Non-Infected	19.89 ± 0.1 ^a	21.9 ± 0.08 ^a	23.39 ± 0.3 ^a	26.64 ± 0.1 ^a	32.92 ± 0.1 ^a	42.33 ± 0.4 ^a
	Infected	18.34 ± 0.4 ^c	21.3 ± 0.32 ^b	22.61 ± 0.2 ^b	25.10 ± 0.1 ^c	31.35 ± 0.4 ^b	40.96 ± 0.5 ^b
Number of leaves							
ICPL 11260	Non-Infected	5.33 ± 0.6 ^b	8.00 ± 1.0 ^b	10.67 ± 0.6 ^b	14.33 ± 0.6 ^b	17.00 ± 1.0 ^c	23.67 ± 0.6 ^b
	Infected	3.33 ± 0.6 ^d	5.67 ± 0.6 ^d	8.33 ± 0.6 ^c	8.67 ± 1.2 ^c	11.33 ± 1.2 ^d	13.00 ± 1.0 ^d
IPAC-02	Non-Infected	7.00 ± 1.0 ^a	9.33 ± 1.2 ^a	13.0 ± 1.0 ^a	18.33 ± 0.6 ^a	22.33 ± 0.6 ^a	25.67 ± 0.6 ^a
	Infected	4.33 ± 0.6 ^c	7.00 ± 1.0 ^c	10.67 ± 0.6 ^b	14.33 ± 0.6 ^b	19.00 ± 1.0 ^b	22.67 ± 1.2 ^c
Fresh weight (g)							
ICPL 11260	Non-Infected	0.50 ± 0.05 ^b	1.06 ± 0.06 ^b	2.20 ± 0.08 ^a	3.45 ± 0.12 ^a	4.09 ± 0.08 ^b	5.12 ± 0.08 ^a
	Infected	0.39 ± 0.04 ^d	0.54 ± 0.08 ^d	1.14 ± 0.07 ^b	1.42 ± 0.06 ^c	2.15 ± 0.12 ^d	2.74 ± 0.18 ^c
IPAC-02	Non-Infected	0.62 ± 0.04 ^a	1.14 ± 0.06 ^a	2.27 ± 0.09 ^a	3.56 ± 0.10 ^a	4.30 ± 0.17 ^a	5.26 ± 0.10 ^a
	Infected	0.47 ± 0.04 ^c	0.68 ± 0.09 ^c	1.19 ± 0.09 ^b	2.57 ± 0.12 ^b	3.13 ± 0.08 ^c	3.82 ± 0.13 ^b
Dry weight (g)							
ICPL 11260	Non-Infected	0.17 ± 0.02 ^b	0.21 ± 0.02 ^a	0.67 ± 0.05 ^b	0.95 ± 0.02 ^a	0.90 ± 0.05 ^b	1.13 ± 0.03 ^b
	Infected	0.11 ± 0.04 ^c	0.15 ± 0.05 ^c	0.44 ± 0.02 ^c	0.39 ± 0.04 ^c	0.48 ± 0.04 ^d	0.71 ± 0.04 ^d
IPAC-02	Non-Infected	0.35 ± 0.21 ^a	0.22 ± 0.05 ^a	0.82 ± 0.03 ^a	0.95 ± 0.03 ^a	1.01 ± 0.04 ^a	1.25 ± 0.02 ^a
	Infected	0.17 ± 0.03 ^b	0.19 ± 0.05 ^b	0.61 ± 0.04 ^b	0.71 ± 0.04 ^b	0.80 ± 0.02 ^c	1.03 ± 0.02 ^c

Data are represented as means of three replicates (±SD). Different letters (a, b, c, d) within each column indicate significant differences between groups at $p < 0.05$ based on Duncan's multiple range test.

Shoot length showed strong main effects of variety ($F_{1,48} = 728.71$, $p < 0.001$, $\eta^2 = 0.938$), treatment ($F_{1,48} = 871.89$, $p < 0.001$, $\eta^2 = 0.948$), and DAI ($F_{5,48} = 1201.49$, $p < 0.001$, $\eta^2 = 0.992$). All interaction terms were significant, including variety × treatment ($F_{1,48} = 573.89$, $p < 0.001$), variety × DAI ($F_{5,48} = 61.68$, $p < 0.001$), treatment × DAI ($F_{5,48} = 68.92$, $p < 0.001$), and the three-way interaction ($F_{5,48} = 53.66$, $p < 0.001$). The MSE for shoot length was 0.483.

For number of leaves, significant effects were observed for variety ($F_{1,48} = 248.53$, $p < 0.001$, $\eta^2 = 0.838$), treatment ($F_{1,48} = 272.00$, $p < 0.001$, $\eta^2 = 0.850$), and DAI ($F_{5,48} = 478.48$, $p < 0.001$, $\eta^2 = 0.980$). Significant interaction effects were detected for variety × treatment ($F_{1,48} = 19.06$, $p < 0.001$), variety × DAI ($F_{5,48} = 17.99$, $p < 0.001$), treatment × DAI ($F_{5,48} = 12.02$, $p < 0.001$), and the three-way interaction ($F_{5,48} = 7.06$, $p < 0.001$). The MSE for number of leaves was 0.944.

Fresh weight was significantly affected by variety ($F_{1,48} = 70.81$, $p < 0.001$, $\eta^2 = 0.596$), treatment ($F_{1,48} = 789.61$, $p < 0.001$, $\eta^2 = 0.943$), and DAI ($F_{5,48} = 986.02$, $p < 0.001$, $\eta^2 = 0.990$). All interaction effects were significant, including the three-way interaction ($F_{5,48} = 6.08$, $p < 0.001$). The MSE for fresh weight was 0.027.

Dry weight also showed significant main effects of variety ($F_{1,48} = 156.70$, $p < 0.001$, $\eta^2 = 0.766$), treatment ($F_{1,48} = 125.90$, $p < 0.001$, $\eta^2 = 0.724$), and DAI ($F_{5,48} = 183.90$, $p < 0.001$, $\eta^2 = 0.950$). Significant interaction effects were observed for variety × treatment ($F_{1,48} = 34.74$, $p < 0.001$), variety × DAI ($F_{5,48} = 5.47$, $p < 0.001$), treatment × DAI ($F_{5,48} = 7.04$, $p < 0.001$), and variety × treatment × DAI ($F_{5,48} = 7.67$, $p < 0.001$). The MSE for dry weight was 0.008.

3.3 Effect of Infection of *P. cajani* on Anatomical Responses of Pigeon Pea Varieties

Roots: Transverse sections of non-infected ICPL 11260 and IPAC-02 roots on the 15th day after infection exhibited 2–4 layers of cork, several layers of cortex, a layer of endodermis, a few patches of pericyclic fibers, and centrally located xylem and xylem parenchyma, with phloem situated just above the xylem. By the 30th day, non-infected roots developed secondary structures, including a cambium ring, secondary xylem, and xylem vessels. In infected ICPL 11260, mycelial growth was evident at both 15 and 30 days after infection. On the 15th day, epidermal cells were extensively damaged, and the cortex was disorganized, with xylem and phloem vessels attacked. By the 30th day, roots showed widespread mycelial invasion. In contrast, infected IPAC-02 roots remained free from pathogen invasion, displaying well-organized secondary xylem and phloem vessels, with dark purple staining due to lignification of the cell walls (Fig. 1).

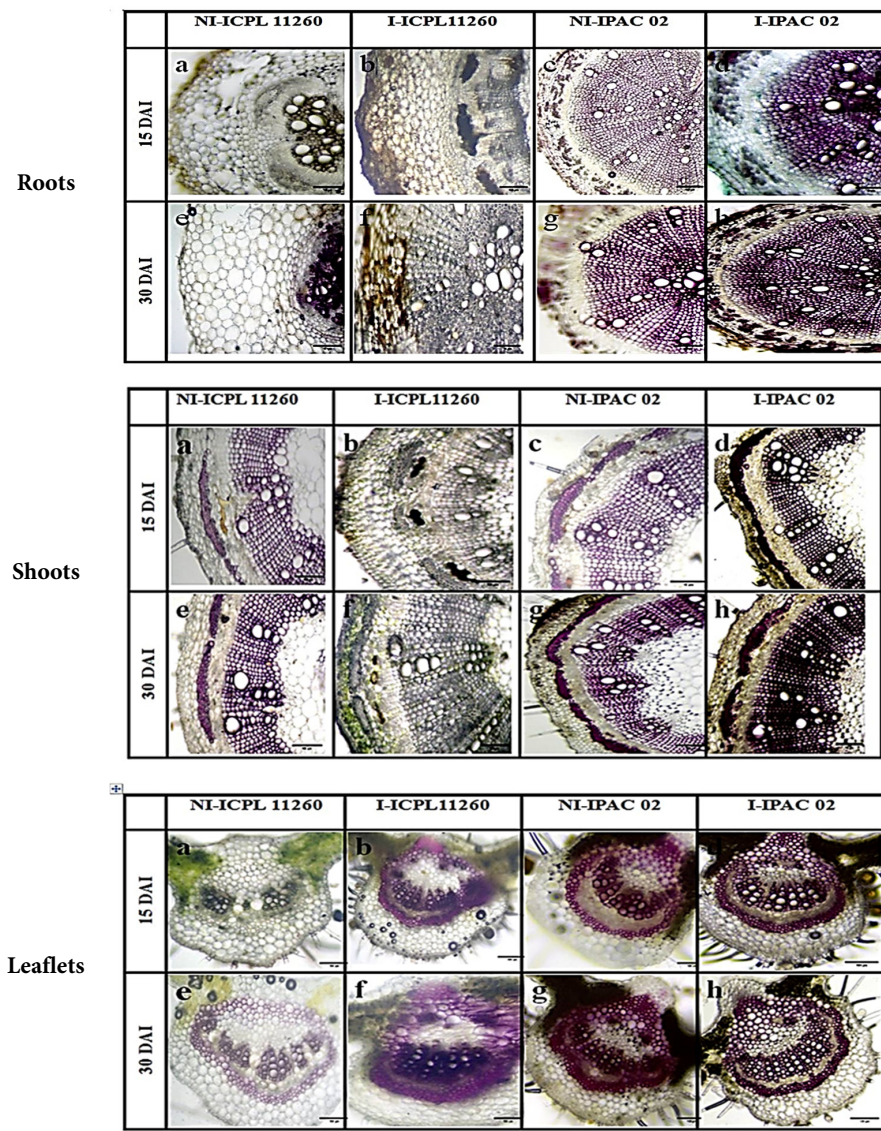


Figure 1: Transverse section of both infected and non-infected pigeon pea plant roots, shoots, and leaflets on the 15th and 30th days after infection (DAI), 10× and 100 μm scale bar. (a—non-infected ICPL 11260 at 15th DAI, b—infected ICPL 11260 at 15th DAI, c—non-infected IPAC-02 at 15th DAI, d—infected IPAC-02 at 15th DAI, e—non-infected ICPL 11260 at 30th DAI, f—infected ICPL 11260 at 15th DAI, g—non-infected IPAC-02 at 30th DAI, h—infected IPAC-02 at 30th DAI).

Stems: In transverse sections of non-infected ICPL 11260 and IPAC-02 stems, a layered epidermis was observed, followed by a cortical zone, irregular patches of pericyclic fibers, and an open bicollateral vascular bundle. The xylem was radially arranged and occupied the majority of the section, with phloem located just above it, and the centrally located pith gradually rupturing as the plant matured. In infected ICPL 11260 stems, by the 15th day, the pathogen had attacked the cortical region and phloem, progressing toward the pith. By the 30th day, the entire stem was invaded, disrupting the epidermis and cortex, with phloem and xylem vessels covered in mycelia. In contrast, infected IPAC-02 stems showed no pathogen invasion, maintained well-organized cell structures, developed secondary xylem and phloem vessels, and exhibited extensive lignification of cell walls (Fig. 1).

Leaflets: Transverse sections of non-infected ICPL 11260 and IPAC-02 leaflets through the midrib exhibited a single-layered upper and lower epidermis, often interrupted by trichomes, with collenchyma beneath the epidermis on both sides. The centrally located open bicollateral vascular bundle, consisting of xylem and phloem, was supported by ground tissue and surrounded by a pericyclic fiber ring. In infected ICPL 11260, 15 days after infection, the mesophyll was affected and tissues darkened, although the epidermis remained largely intact. By 30 days, the entire leaf was invaded, with disruption of the epidermis and phloem and xylem vessels covered with mycelia. Infected IPAC-02 showed no signs of pathogen invasion at any stage; instead, cell structures remained well-organized, secondary phloem and xylem vessels were formed, and extensive lignification of cell walls was observed (Fig. 1).

Overall, these anatomical observations indicate that ICPL 11260 is highly susceptible to *P. cajani*, exhibiting extensive structural damage across roots, stems, and leaves, whereas IPAC-02 demonstrates structural resilience likely associated with defense responses such as lignification and maintenance of organized vascular tissues.

3.4 Biochemical Studies

3.4.1 Effect of Infection of *P. cajani* on Osmolytes of Pigeon Pea Varieties

The data in Table 3 show that osmolyte levels (total soluble proteins and total soluble sugars) increased significantly with increasing days after infection by *P. cajani* in both pigeon pea varieties. The most pronounced increases in both parameters were observed at 35 days after infection in both non-infected and infected plants compared with pre-infection values. The resistant variety (IPAC-02) exhibited higher levels of total soluble proteins and sugars in infected plants than the susceptible variety (ICPL 11260). In ICPL 11260, infection caused a significant decrease in total soluble proteins and sugars compared with the non-infected control. Conversely, in IPAC-02, infection resulted in a significant increase in both parameters compared with the corresponding non-infected plants. Among all treatments, infected IPAC-02 at 35 days after infection recorded the highest total soluble sugars (153.73 mg/g FW) and total soluble proteins (25.43 mg/g FW), whereas infected ICPL 11260 at the same time exhibited the lowest levels of total soluble sugars (75.41 mg/g FW) and total soluble proteins (22.70 mg/g FW).

3.4.2 Effect of Infection of *P. cajani* on Secondary Metabolites of Pigeon Pea Varieties

The results shown in Table 3 demonstrate that the levels of secondary metabolites (total phenols and total flavonoids) increased significantly with the number of days following *P. cajani* infection in both pigeon pea varieties. Compared with pre-infection values, the most pronounced increases in total phenols and total flavonoids were observed after 35 days of infection in both infected and non-infected plants. In both the susceptible variety (ICPL 11260) and the resistant variety (IPAC-02), infected plants exhibited significantly higher levels of total phenols and total flavonoids than their respective non-infected controls.

At 35 days after infection, infected IPAC-02 recorded the highest total phenol content (51.74 mg/g FW) and total flavonoid content (33.11 mg/g FW), whereas infected ICPL 11260 showed the lowest levels of total phenols (34.23 mg/g FW) and total flavonoids (15.93 mg/g FW).

The higher accumulation of phenolic compounds in the resistant variety is consistent with the anatomical observations in the present study, which showed enhanced lignification in the roots, stems, and leaves of IPAC-02. Since phenols serve as precursors for lignin biosynthesis, their elevated levels likely contribute to the reinforcement of secondary cell wall structures surrounding infection sites. Moreover, phenolic compounds act as key signaling molecules that activate multiple defense-related pathways, including systemic acquired resistance (SAR), thereby enhancing overall resistance to *P. cajani*.

Table 3: Effect of *P. cajani* infestation on osmolytes and secondary metabolites content of pigeon pea varieties.

Pigeon Pea Varieties	Treatment	Days after Infection (DAI)						
		Pre-Infection	10	15	Total Soluble Protein (mg/g FW)			
ICPL11260	Non-Infected	14.38 ± 0.3 ^b	15.06 ± 0.1 ^c	15.86 ± 0.1 ^b	16.34 ± 0.2 ^c	18.59 ± 0.1 ^c	22.9 ± 0.2 ^a	25.85 ± 0.2 ^a
	Infected	10.05 ± 0.2 ^d	11.36 ± 0.1 ^d	13.77 ± 0.1 ^c	14.58 ± 0.2 ^d	18.43 ± 0.1 ^d	19.7 ± 0.3 ^d	22.70 ± 0.2 ^d
IPAC-02	Non-Infected	16.01 ± 0.3 ^b	17.00 ± 0.2 ^b	17.86 ± 0.1 ^b	19.52 ± 0.2 ^b	19.07 ± 0.1 ^b	20.0 ± 0.3 ^c	22.97 ± 0.2 ^c
	Infected	19.52 ± 0.4 ^a	20.39 ± 0.2 ^a	22.39 ± 0.1 ^a	25.02 ± 0.2 ^a	19.36 ± 0.1 ^a	22.4 ± 0.5 ^b	25.43 ± 0.2 ^b
Total soluble sugars (mg/g FW)								
ICPL11260	Non-Infected	45.66 ± 0.5 ^c	48.84 ± 0.3 ^b	50.11 ± 0.4 ^b	77.69 ± 0.6 ^b	84.48 ± 0.5 ^b	93.0 ± 0.8 ^b	95.97 ± 0.5 ^b
	Infected	45.59 ± 0.5 ^c	48.49 ± 0.3 ^b	47.64 ± 0.4 ^b	73.31 ± 0.8 ^b	59.59 ± 0.4 ^c	72.4 ± 0.9 ^c	75.41 ± 0.6 ^c
IPAC-02	Non-Infected	48.77 ± 0.6 ^a	49.55 ± 0.4 ^b	51.24 ± 0.5 ^b	58.74 ± 0.4 ^c	81.58 ± 0.7 ^b	89.4 ± 1.0 ^b	92.41 ± 0.8 ^b
	Infected	48.34 ± 0.6 ^b	73.61 ± 0.5 ^a	138.23 ± 0.8 ^a	127.27 ± 0.9 ^a	149.6 ± 1.0 ^a	150.7 ± 1.2 ^a	153.73 ± 0.9 ^a
Total phenols (mg catechol/g FW)								
ICPL 11260	Non-Infected	27.48 ± 0.1 ^c	33.89 ± 0.3 ^b	37.39 ± 0.1 ^b	39.36 ± 0.2 ^b	39.59 ± 0.2 ^b	39.95 ± 0.2 ^b	42.95 ± 0.4 ^b
	Infected	28.73 ± 0.1 ^b	33.04 ± 0.2 ^c	28.73 ± 0.1 ^c	39.05 ± 0.3 ^b	35.19 ± 0.2 ^c	31.23 ± 0.2 ^d	34.23 ± 0.5 ^d
IPAC-02	Non-Infected	29.35 ± 0.1 ^a	32.48 ± 0.3 ^d	36.08 ± 0.2 ^b	34.20 ± 0.3 ^c	38.33 ± 0.4 ^b	37.17 ± 0.3 ^c	40.17 ± 0.3 ^c
	Infected	29.35 ± 0.1 ^a	51.56 ± 0.2 ^a	45.46 ± 0.2 ^a	54.53 ± 0.4 ^a	52.84 ± 0.4 ^a	48.74 ± 0.3 ^a	51.74 ± 0.5 ^a
Total flavonoids (mg quercetin/g FW)								
ICPL 11260	Non-Infected	5.00 ± 0.01 ^d	5.60 ± 0.01 ^d	7.12 ± 0.02 ^c	7.71 ± 0.01 ^d	11.93 ± 0.2 ^d	13.85 ± 0.2 ^{bc}	16.85 ± 0.05 ^{bc}
	Infected	6.76 ± 0.01 ^c	5.98 ± 0.02 ^c	5.95 ± 0.01 ^d	9.69 ± 0.01 ^c	12.52 ± 0.2 ^c	14.90 ± 0.2 ^b	17.90 ± 0.05 ^b
IPAC-02	Non-Infected	8.63 ± 0.01 ^b	6.21 ± 0.02 ^b	8.23 ± 0.02 ^b	14.68 ± 0.02 ^b	15.25 ± 0.2 ^b	12.93 ± 0.5 ^c	15.93 ± 0.04 ^c
	Infected	10.71 ± 0.01 ^a	6.66 ± 0.02 ^a	11.56 ± 0.03 ^a	18.69 ± 0.03 ^a	26.13 ± 0.4 ^a	30.10 ± 0.8 ^a	33.10 ± 0.03 ^a

Data are represented as means of three replicates (±SD). Different letters (a, b, c, d) within each column indicate significant differences between groups at $p < 0.05$ based on Duncan's multiple range test.

3.4.3 Effect of Infection of *P. cajani* on Pathogenesis-Related Proteins of Pigeon Pea Varieties

The data presented in Table 4 show that the levels of pathogenesis-related proteins— β -1,3-glucanases and chitinases—rose significantly with increasing days after *P. cajani* infection in both pigeon pea varieties. The most pronounced increases in both enzymes were observed 35 days after infection when compared with the pre-infection baseline. Across all sampling intervals, the resistant variety (IPAC-02) accumulated markedly higher amounts of β -1,3-glucanases and chitinases in infected plants than the susceptible variety (ICPL 11260). In ICPL 11260, enzyme activities in infected tissues decreased significantly relative to non-infected controls, indicating a suppressed or ineffective defense response. In contrast, infected IPAC-02 plants exhibited significant increases in both enzymes compared to their non-infected counterparts, reflecting a strong activation of defense pathways.

Quantitatively, infected IPAC-02 plants at 35 days after infection recorded the highest chitinase (11.43 U mg⁻¹ protein) and β -1,3-glucanase (9.09 U mg⁻¹ protein) activities. Conversely, infected ICPL

11260 plants at the same time point showed the lowest activities of both defense-related enzymes, with chitinase at 4.56 U mg^{-1} protein and substantially reduced β -1,3-glucanase levels. These findings highlight the more robust enzymatic defense machinery in the resistant genotype, contributing to its enhanced tolerance against *P. cajani*.

3.5 Two Way Anova of Biochemical Analysis

Normality of residuals was assessed using the Shapiro–Wilk test and Q–Q plots. Homogeneity of variances was verified using Levene’s test, which was satisfied for total soluble protein, sugars, phenols, and flavonoids ($p > 0.05$), while β -glucanase and chitinase showed heteroscedasticity ($p < 0.05$). Given the balanced experimental design, ANOVA was considered robust and was applied to all variables.

Table 4: Effect of *P. cajani* infestation on pathogenesis-related proteins (β -1,3-Glucanases and chitinases) of pigeon pea varieties.

Pigeon Pea Varieties	Treatment	Days after Infection (DAI)						
		Pre-Infection	10	15	β -1,3-Glucanases (U. mg^{-1} Protein)			
					20	25	30	35
ICPL 11260	Non-Infected	1.35 ± 0.01^c	2.17 ± 0.02^b	2.55 ± 0.02^b	2.78 ± 0.01^b	3.33 ± 0.02^c	3.41 ± 0.01^c	6.41 ± 0.04^c
	Infected	1.29 ± 0.01^d	1.51 ± 0.02^c	1.07 ± 0.02^c	1.40 ± 0.01^c	1.70 ± 0.02^d	1.90 ± 0.01^d	4.90 ± 0.05^d
IPAC-02	Non-Infected	1.44 ± 0.01^a	2.06 ± 0.02^b	2.48 ± 0.02^b	3.14 ± 0.01^b	3.97 ± 0.03^b	3.90 ± 0.01^b	6.90 ± 0.05^b
	Infected	1.40 ± 0.01^b	3.77 ± 0.02^a	5.56 ± 0.02^a	8.83 ± 0.02^a	7.23 ± 0.04^a	6.09 ± 0.02^a	9.09 ± 0.05^a
		Chitinases (U. mg^{-1} protein)						
ICPL 11260	Non-Infected	3.51 ± 0.01^b	3.77 ± 0.02^c	4.00 ± 0.02^c	4.33 ± 0.01^b	4.71 ± 0.03^b	4.99 ± 0.02^b	7.99 ± 0.06^b
	Infected	3.52 ± 0.01^b	3.24 ± 0.02^d	2.60 ± 0.03^d	1.22 ± 0.01^c	2.01 ± 0.03^c	1.56 ± 0.02^c	4.56 ± 0.06^c
IPAC-02	Non-Infected	3.37 ± 0.01^c	4.05 ± 0.03^b	4.41 ± 0.03^b	4.25 ± 0.02^b	4.81 ± 0.04^b	4.71 ± 0.03^b	7.71 ± 0.04^b
	Infected	3.98 ± 0.01^a	7.19 ± 0.03^a	8.04 ± 0.04^a	8.64 ± 0.04^a	8.89 ± 0.01^a	8.43 ± 0.05^a	11.43 ± 0.05^a

Data are represented as means of three replicates (\pm SD). Different letters (a, b, c, d) within each column indicate significant differences between groups at $p < 0.05$ based on Duncan’s multiple range test.

Factorial ANOVA revealed significant main effects on all defense-related biochemical parameters examined. Total soluble protein ($F_{6,56} = 98.99$, $p < 0.001$, $\eta^2 = 0.914$), total soluble sugars ($F_{6,56} = 7430.14$, $p < 0.001$, $\eta^2 = 0.999$), phenolic content ($F_{6,56} = 178.62$, $p < 0.001$, $\eta^2 = 0.950$), and flavonoid content ($F_{6,56} = 457.47$, $p < 0.001$, $\eta^2 = 0.980$) exhibited pronounced time-dependent responses, indicating strong modulation of primary and secondary metabolites during disease progression. Defense-related enzyme activities were significantly influenced by genotype and treatment; chitinase activity differed markedly between pigeon pea varieties ($F_{1,56} = 198.46$, $p < 0.001$, $\eta^2 = 0.780$), demonstrating a strong genotype-dependent defense response, whereas β -glucanase activity was significantly affected by infection treatment ($F_{1,56} = 203.92$, $p < 0.001$, $\eta^2 = 0.785$), reflecting pathogen-induced enzyme activation.

Factorial ANOVA revealed significant main effects on all defense-related biochemical parameters examined. Total soluble protein, sugars, phenols, and flavonoids exhibited pronounced time-dependent responses ($p < 0.001$), with large effect sizes ($\eta^2 \geq 0.91$). Defense-related enzyme activities were significantly influenced by genotype and treatment, with chitinase showing a strong varietal effect and β -glucanase responding significantly to infection treatment ($p < 0.001$).

Mean values are presented with Data were analyzed using factorial analysis of variance (ANOVA) to evaluate the effects of variety, treatment, and days after infection (DAI) on morphological and biochemical parameters. Prior to analysis, assumptions of ANOVA were assessed by examining the normality of residuals using the Shapiro–Wilk test and homogeneity of variances using Levene’s test. While some morphological and enzyme-related parameters showed deviations from normality and homoscedasticity ($p < 0.05$), the

balanced experimental design with equal replication across treatments and time points ensured robustness of ANOVA. Effect sizes were expressed as partial eta squared (η^2). Mean separation was performed using Tukey's HSD test at $p \leq 0.05$. Standard errors correspond to 95% confidence intervals.

4 Discussion

Pigeon pea is one of the most important legume crops grown in subtropical and tropical regions, yet its productivity is severely constrained by a range of biotic stressors. Ensuring a sustainable food supply for an expanding global population requires mitigating the significant yield losses caused by these stresses. Among them, *Phytophthora* blight is recognized as one of the most destructive diseases affecting pigeon pea cultivation [21]. The present morphological analyses demonstrated that infection with *Phytophthora drechsleri* f. sp. *cajani* caused substantial reductions in key growth parameters—including root length, shoot length, number of leaves, fresh weight, and dry weight—across both pigeon pea varieties. In the susceptible genotype ICPL 11260, pathogen infection resulted in a drastic (0.5- to 2-fold) decline in all growth traits compared with non-infected controls, suggesting that the pathogen was able to establish and proliferate effectively within host tissues.

The higher dry weight observed in infected plants may be attributed to the formation of secondary structural components, increased lignification, and the synthesis of various defense-related metabolites. Conversely, the greater fresh weight recorded in non-infected IPAC-02 may reflect its superior water uptake capacity, which appears compromised in infected plants. Fungal invasion is known to disrupt normal physiological processes, as plants redirect metabolic activity towards defense, often at the expense of growth. Nevertheless, both non-infected ICPL 11260 and IPAC-02 exhibited steady, continuous growth throughout the experimental period. Overall, the results clearly indicate that *Phytophthora* infection leads to a marked decline in all measured growth traits.

These findings align with earlier reports. Ansari et al. [22] documented similar reductions in stem length, root length, and leaf number in tomato plants infected with *Fusarium*, highlighting the broader impact of fungal pathogens on vegetative development. Likewise, Yadav and Jha [21] observed significant decreases in growth parameters of pigeon pea varieties artificially inoculated with *Phytophthora* zoospore suspensions under favorable disease conditions. Their study reported pronounced reductions in growth traits in infected varieties—particularly Shrawani and ICPL 11260—after 30 days of infection. Minor variations in leaf number observed at 20 days post-infection in ICPL 11260 and IPAC-02 were attributed to the relatively slower progression of the pathogen toward leaf tissues. They further noted that varieties such as Shweta and ICPL 11260 exhibited partial defense responses capable of slowing pathogen advancement.

Yadav and Jha [21] emphasized that pathogen infection often reduces plant growth and reproductive capacity because maintaining defense responses is metabolically expensive. Moreover, many fungal pathogens withdraw nutrients directly from host tissues and produce non-enzymatic toxins that alter host physiology, reducing resource availability and ultimately suppressing growth in susceptible varieties.

The transverse sections of roots, stems, and leaves of healthy plants exhibited normal anatomical organization with intact epidermal layers, well-differentiated cortical tissues, and clearly defined vascular elements. In contrast, infected ICPL 11260 plants showed extensive cellular damage and tissue disruption resulting from fungal invasion. Mycelial growth was evident in the epidermal and cortical regions, accompanied by degradation and collapse of secondary structures such as phloem and xylem vessels. These disruptions became more pronounced by 30 days after infection, when mycelia had progressed from the epidermis into the vascular tissues and eventually toward the pith, ultimately leading to complete plant mortality.

Infected IPAC-02 plants, however, maintained well-organized cellular architecture, and no pathogen penetration or mycelial proliferation was observed at any stage. Prominent lignification of cell walls was detected throughout the tissues, indicating a strong and early activation of defense responses in the resistant variety. The enhanced lignification observed in roots, stems, and leaves of IPAC-02 aligns with the elevated phenolic content measured in this genotype. Phenols serve as precursors for lignin biosynthesis and act as key signaling molecules that activate multiple defense-related genes and pathways, including systemic acquired resistance (SAR). Their incorporation into secondary cell walls strengthens the tissues surrounding infection sites, effectively reducing pathogen spread. These findings are supported by earlier studies, which identify cell wall fortification through lignification as a primary determinant of host resistance [23]. Following initial pathogen penetration, resistant plants typically undergo rapid biochemical and anatomical modifications—such as lignin deposition—that reinforce the cell wall and restrict the activity of fungal enzymes [24].

Both varieties showed increased total soluble proteins and sugars following infection, but the magnitude of increase was significantly higher in IPAC-02. Osmolytes play critical roles in maintaining cellular homeostasis, stabilizing proteins, regulating osmotic balance, and supporting metabolic activity under stress. The sharp decline in protein and sugar levels in infected ICPL 11260 suggests impaired metabolic capacity, likely due to pathogen-induced cellular damage. In contrast, IPAC-02 displayed enhanced osmolyte accumulation, which may have supported its ability to maintain turgor pressure, cell wall strengthening, and active defense responses during pathogen invasion [25].

Both total phenols and flavonoids increased significantly after infection, with the highest concentrations observed at 35 days. Phenolic compounds are directly linked to lignin biosynthesis and contribute to the reinforcement of secondary walls around infection sites. The resistant variety exhibited substantially higher phenol and flavonoid levels than the susceptible variety, a pattern consistent with strong induced systemic resistance. Phenolics also function as antimicrobial agents and signaling molecules that activate defense pathways such as SAR. The strong correlation between phenol accumulation and enhanced lignification observed in IPAC-02 supports earlier findings by Zhou et al. [26] and Rathod et al. [24].

IPAC-02 showed pronounced increases in β -1,3-glucanase and chitinase activity, while ICPL 11260 showed a significant decline. PR proteins contribute to host resistance by degrading fungal cell wall components, releasing elicitor molecules, and triggering additional defense pathways. The higher PR protein activity in IPAC-02 suggests an efficient recognition of fungal infection and a rapid activation of defense cascades. Similar declines in glucanase activity in susceptible hosts have been reported in avocados infected with *Phytophthora cinnamomi* [27]. Likewise, pigeon pea plants infected with Fusarium wilt displayed reduced phenol, β -glucanase, and chitinase activities in susceptible lines compared to resistant lines [28].

The induction of pathogenesis-related (PR) proteins during the early stages of pathogen infections is a key plant defense mechanism against invasion. When pathogens invade, the plant enzyme beta-1,3-glucanase is activated. This enzyme catalyzes the hydrolysis of the pathogen's hyphal cell wall, leading to the release of beta-1,3-glucan oligosaccharides. These oligosaccharides act as elicitors and microbe-associated molecular patterns, which trigger the activation of signaling cascades [29,30]. This process ultimately results in the production of antimicrobial compounds, such as phytoalexins, which contribute to a range of local and systemic defense responses, helping to prevent the progression of the pathogen within the plant [21]. Chitinases are part of the glycosyl hydrolase family, which catalyze the hydrolysis of chitin glycosidic bonds. These enzymes were among the first proteins recognized for their significant role in innate immunity. They catalyze the breakdown of chitin, a component of fungal cell

walls, which, upon hydrolysis, acts as a biological elicitor. This process sends signals to cell membrane receptors, triggering defense mechanisms. Additionally, the peroxidase (POD) enzyme plays a crucial role in either activating these defense mechanisms or alleviating the negative effects of oxidative stress during a pathogen attack [21].

The increased activity of beta-glucanase and chitinase in the resistant plants is due to the signaling response by the MAPK cascade during pathogen attack, which activates PR genes, resulting in the synthesis of the chitinases/ β -1, 3-glucanases that cause systemic acquired resistance [31]. Total phenol contents increase in resistant varieties because, during the infection, the plant undergoes various alterations in its cellular structure and morphological level, which are ultimately induced by the response of overregulation of several phenolic compounds [32,33]. According to Hassan et al. [34], basal resistance, which is brought on by an infection's assault, can help to explain the increase in the total phenol and flavonoid contents after infection in the resistant plants. Resistant plant cells get strengthened against pathogen attacks by collecting compounds such as flavonoids and phenolic acids when they recognize the microbe-associated molecular patterns (MAMPs) as a part of early and rapid activation of the defense mechanism, which results in the formation of chemical and physical barriers against pathogen progression [35].

5 Conclusions

The combined morphological, anatomical, and biochemical results clearly demonstrate that *Phytophthora* blight imposes severe constraints on the physiological processes and overall growth of pigeon pea, with far greater impact on the susceptible variety ICPL 11260 than on the resistant variety IPAC-02. By the thirty-first day post-infection, ICPL 11260 exhibited marked reductions in fresh and dry biomass, shoot and root length, and leaf number, accompanied by extensive cellular disintegration in root, stem, and leaf tissues. These structural disruptions indicate successful pathogen establishment and systemic spread within susceptible tissues. In contrast, the resistant variety IPAC-02 maintained normal tissue organization and displayed significantly stronger biochemical defense responses, including elevated levels of total soluble proteins, sugars, phenols, and flavonoids, as well as enhanced activity of defense-related enzymes such as chitinase and β -1,3-glucanase. The substantial increase in PR-protein activity in IPAC-02 reflects a more efficient activation of defense signaling pathways, likely contributing to a more robust systemic acquired resistance (SAR) response. Enhanced phenolic content and pronounced lignification further underscore the activation of structural defense mechanisms that restrict pathogen penetration and limit the spread of mycelia within vascular tissues. Collectively, these responses demonstrate the ability of IPAC-02 to mount a coordinated biochemical and anatomical defense that confers strong resistance to *P. cajani*. Importantly, this study presents the first integrated assessment combining morphological, anatomical, and biochemical traits to differentiate susceptible and resistant pigeon pea genotypes under *P. cajani* infection. The quantitative association between lignification, secondary metabolite accumulation, and PR-protein activation provides a set of reliable biological markers for identifying resistant genotypes. These findings offer valuable insights for breeding programs aimed at developing *Phytophthora*-resistant pigeon pea cultivars and contribute to efforts toward sustainable legume production under biotic stress.

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Abbreviations

DAI	days after infection
DMAB	p-dimethylaminobenzaldehyde
MAMPs	microbe-associated molecular patterns
MAPK	mitogen-activated protein kinase
POD	peroxidase
PPO	polyphenol oxidase
PAL	phenylalanine ammonia lyase
PR	pathogenesis-related proteins
SAR	systemic acquired resistance

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