

First report of *Fusarium nygamai* causing pigeon pea wilt across Uttar Pradesh

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Abstract

Fusarium wilt is a destructive vascular disease that significantly affects pigeonpea production in different regions. In this study, *Fusarium* strains were isolated and characterised from wilt-affected pigeonpea plants from 16 districts of Uttar Pradesh, India. Of the 97 isolates, five strains showed higher pathogenicity with respect to pectate lyase and cellulase activities and were further studied *in vitro* and *in vivo*. *Fusarium* spp. were confirmed by molecular identification using internal transcribed spacer (*ITS*) and *TEF1- α* gene sequencing, along with phylogenetic analysis. Pathogenicity assays showed that *Fusarium nygamai* was the most virulent, with the highest disease severity index (63%). Interactions between *Fusarium udum* and combined inoculations of *Fusarium nygamai* and *Fusarium udum* were observed to compare the pathogenicity levels among the strains. This is the first report of *Fusarium nygamai* causing wilt in pigeonpeas, highlighting its increasing importance as a major pathogen. The results will serve as baseline information for understanding the cause of *Fusarium* wilt and devising suitable strategies for controlling the disease in pigeonpeas.

Highlights

- First report of causing vascular wilt in pigeonpea.
- Five out of 97 isolates showed high PL and CL activity.
- showed higher virulence than in pigeonpea.
- and co-infection increased disease severity.

1. Introduction

Pigeonpea (*Cajanus cajan*) belongs to the Fabaceae (Leguminosae) family, which is one of the six important leguminous crops grown globally, along with common beans, cowpeas, field peas, lentils, and chickpeas. It serves as a significant source of food and nutritional security for millions of farmers, especially in countries such as Africa, Southeast Asia, and America (Varshney et al. 2017). Pigeonpea crops are important because they contain high protein, essential amino acids, minerals such as calcium and iron, and water-soluble vitamins such as thiamine, riboflavin, niacin, and vitamin C (Ojwang et al. 2021). Furthermore, the bioactive compounds in pigeon peas have antidiabetic, anti-inflammatory, and antioxidant properties; absence of saturated fat, sodium, and cholesterol; and low levels of saturated fat, which are beneficial for sustainable agriculture (Dinore et al. 2022). Globally, pigeonpea production amounts to approximately 5 million tons, cultivated on 6.1 million hectares of land. India is responsible for 80% of the global production and utilises 67% of the total land area dedicated to this crop (Patidar et al. 2024), and it is cultivated in all states of the country. The primary states for pigeonpea cultivation in India are Maharashtra, Karnataka, Madhya Pradesh, Uttar Pradesh, Gujarat, Jharkhand, Telangana, and Andhra Pradesh (Saxena et al. 2024). Despite its significance as a food source, this pulse crop has not yet received sufficient recognition and is adversely affected by various economically devastating fungal,

viral, and bacterial diseases, such as *Fusarium* wilt, sterility mosaic, and Phytophthora. *Fusarium* wilt is the most common and severe disease affecting cotton. *Fusarium* wilt is a vascular wilt disease caused by *Fusarium oxysporum*, a widespread and diverse fungal species that includes both pathogenic and non-pathogenic strains (Leslie et al. 2006). Pathogenic strains are host-specific and lead to yield reductions in several significant crops, such as tomatoes, cucumbers, melons, flax, lettuce, strawberries, oil palms, tobacco, carnations, cotton, and bananas (Leslie et al. 2006). This disease can result in considerable losses in crop growth and productivity, with incidence rates ranging from 30% to 60% during the flowering to maturity stages (Sharma et al. 2016). *Fusarium oxysporum* is an anamorphic fungus that lacks sexual stages (Zhang et al. 2019). It produces three types of asexual spores: microconidia, macroconidia, and chlamydospores. While the survival of conidia and mycelia in the soil is brief, chlamydospores persist longer and are regarded as the primary means of *F. oxysporum* persistence in the soil (Tian et al. 2021). *Fusarium* wilt is a prevalent and destructive disease that is challenging to manage, leading to significant yield and quality losses, and poses a major constraint to the sustainable production of pigeonpea (B. M et al. 2022), has reported pathogenic variability of 60 *Fusarium udum* isolates from diverse geographical locations against 11 pigeonpea host. In addition, *Fusarium* wilt in chilli comprises three species, viz. *Fusarium oxysporum*, *Fusarium solani*, and *Fusarium equiseti*, with *F. equiseti* being identified for the first time (Hami A et al. 2021). Interactions between these two pathogens cause more damage and higher wilt severity than the sum of the damage caused by each agent alone individually. In addition, the alternaria blight in pigeonpea was identified as *A. tenuissima*, while *A. alternata* has also been reported for the same disease (Balai et al. 2016). Root rot in garden peas caused by the *Fusarium solani* species complex was reported by (Coleman et al. 2016). The pathogens *Fusarium virguliforme*, *F. tucumaniae*, *F. brasiliense*, and *F. cuneirostrum*, which are implicated in soybean sudden death syndrome, belong to the *Fusarium* species complex. The pathogenicity of *F. oxysporum* is attributed to its diverse pathogenicity factors, including secreted effectors, mycotoxins, and cell wall-degrading enzymes (CWDEs), which facilitate host entry and colonisation of the waxy surface (Zuriegat et al. 2021). *Fusarium oxysporum* and related species secrete a range of enzymes, including pectinases (polygalacturonase, pectate lyase, and pectin methylesterase), cellulases, xylanases, β -glucosidases, proteases, and lipases, which depolymerise nearly all major wall components (cellulose, hemicellulose/xylan, pectin, polygalacturonic acids, and wall-associated proteins). These enzymatic activities enable root penetration, movement into the xylem, tissue maceration and nutrient acquisition (Roy et al. 2020). In chickpea wilt, pectinases and xylanases exhibited higher activities in the more virulent race (s), which correlated positively with the development of yellowing and wilting. This indicates their role in the early and late stages of the infection (Perincherry et al. 2021). *Fusarium proliferatum* and *F. oxysporum* mediate the upregulation of the genes and activities of cellulase, β -glucosidase, xylanase, pectinase, and lipase, which selectively degrade the primary and secondary cell walls during infection. Greater CWDE activity is required to attack the host plant, reflecting its adaptation to the host cell wall composition (Paccanaro et al. 2017). Despite advancements in research on *Fusarium* wilt, the aetiology of the disease and the *Fusarium* species complex affecting pigeonpeas, especially *Fusarium* wilt, have not been fully elucidated. Moreover, the number of fungal pathogens associated with this plant is likely to be underestimated. The present study focused on the variability of pathogens responsible for wilt

disease in different districts of Uttar Pradesh. *Fusarium* isolates were characterised based on mycelial mat colour, growth rate, length and breadth of macroconidia, and variability in pathogenic characteristics, such as production of PCDEs, alkalinity factors, and ability to infect the host. Genotype-environment interactions have also been performed to characterise the combined effects of *F. nygamai* and *F. udum* using tolerant and sensitive pigeon pea cultivars.

2. Materials and methods

2.1. Characterization of collected *Fusarium* isolates from infected pigeon pea plants

A survey was conducted across 16 districts of Uttar Pradesh to evaluate the incidence of *Fusarium* wilt in the pigeon pea fields. Samples were collected from these areas for further analysis. To isolate *Fusarium* from the wilted pigeon pea plant samples, root and stem tissues were surface sterilised using 1–2% sodium hypochlorite for 1–2 min, followed by rinsing with sterile water and drying between sterile filter papers. The inner tissues of the infected parts were sectioned vertically, and fragments of the outer tissues were sectioned horizontally. Rose Bengal Agar (RBA) medium was slightly stabbed with xylem/vascular tissues. The plates were incubated at $28 \pm 2^{\circ}\text{C}$ for 7–10 days (Argüelles-Moyao et al. 2024). Fungal mycelial growth was documented, and distinct colonies exhibiting varied morphologies were selected using sterile loop. The colonies were transferred to new potato dextrose agar plates to obtain pure cultures. With appropriate precautions, overgrowth is unlikely. After three successive isolations, pure isolates were obtained from the samples. All subcultures were stored at 4°C with 15% glycerol and at -80°C (Purohit et al. 2017). Pure cultures were preserved in the Division of Microbial Technology at the NBRI, India.

2.2. Pathogenicity assessments

2.2.1. Activity of cell wall hydrolases

Phyto-pathogenic fungi synthesise specific enzymes that degrade plant cell walls, facilitating their invasion into the host. *Fusarium* spp. predominantly produces pectate lyase and cellulase during infections. A crude enzyme extract was prepared from fungal leaf tissues by homogenising them in an extraction buffer composed of 20 mM EDTA, 20 mM cysteine-HCl, and 0.5% Triton X-100 in a 20 mM sodium phosphate buffer at pH 7. Pectate lyase and cellulase activities were quantified as described previously (Sharafaddin et al. 2019; Nautiyal et al. 2013).

2.2.2. In vitro plate assay

Fusarium infection was analysed using a plate assay. Pigeonpea seeds of two different genotypes, wilt-resistant (IPAC-79) and wilt-sensitive (BAHAR), were used in this study. The genotypes were sourced from the Indian Institute of Pulses Research (IIPR) in Kanpur, Uttar Pradesh, India. Seeds were surface sterilised, and pre-germinated seeds were aseptically transferred and sown on water agar (0.8%) plates.

A 9 mm mycelial disc of each actively growing *Fusarium* isolate was placed on the agar, opposite the seeds. The plates were incubated in a culture room at $25 \pm 2^\circ\text{C}$ for seven days (Khairani S et al. 2022). Thereafter, the seedlings were assessed for (i) visual disease symptoms (lesion development, wilting, and tissue discoloration) and (ii) pectin and cellulase activities using standard enzymatic assays.

2.2.3. *In Vivo Plant Assay*

Wilt-sensitive and wilt-resistant pigeonpea genotypes IPAC-79 and BAHAR were monitored under greenhouse conditions, respectively. One-week-old seedlings were inoculated with a 7-day-old PDB-grown culture of the fungus, and the effects on the seedlings were observed for one month. (Bannihatti et al. 2022).

2.3. Identification

Fusarium strains were grown in Czapek Dox Broth (CDB) media and kept at $25 \pm 2^\circ\text{C}$ for 6–7 d (Beccari et al. 2022). The mycelial mat was then filtered and rinsed with sterile, distilled water. Genomic DNA was extracted from the mycelial mass using the Nucleo Spin Plant Mini kit, following the manufacturer's instructions with slight modifications. For molecular analysis, DNA was extracted from the isolates using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The purity and amount of extracted DNA were assessed using gel electrophoresis and a NanoDrop ND 1000 spectrophotometre (Feng et al. 2010). Key virulent strains JnS1, Rj, VnS, Jh, and Fz were selected for sequencing of the ITS and TEF α region PCR amplified (ITS1-50TCC GTA GGT GAA CCT GCG G-30), (ITS4-50 TCCTCCGCTTAT TGA TAT GC-30), and TEF 1 α primers. The PCR conditions were as follows: initial denaturation at $94\text{--}95^\circ\text{C}$ for 3–5 min, followed by approximately 30–35 cycles of denaturation at $94\text{--}95^\circ\text{C}$ for 30 s, annealing at $55\text{--}60^\circ\text{C}$ for 30 s (often optimised around $56\text{--}60^\circ\text{C}$ depending on primer T_m), and extension at 72°C for 30–60 s to amplify the ~ 540–550 bp rDNA fragment, with a final extension at 72°C for 5–10 min using the respective forward and reverse primers (ITS1-50TCC GTA GGT GAA CCT GCG G-30 & (ITS4 -50 TCCTCCGCTTAT TGA TAT GC-30) TEF α (Achari et al. 2023). The PCR products were separated on a 1.5% agarose gel. The PCR-amplified products were then sequenced (Pastrana A et al. 2017).

2.4. Micromorphological assesment

A cross-section of the collar area was excised and examined under a microscope at 10X magnification. Spores and mycelia are observed in the xylem of infected plants (Srivastava et al. 2024). For microscopy, slides were prepared using freshly grown mycelium mounted on clean glass slides, and the mycelia were stained with lactophenol cotton blue, as described by Riddell et al. (Riddell W et al. 1950). The slides were then examined under a microscope at 10X magnification. *Fusarium* cultures were identified based on the observation of micro-and macroconidia (Yang et al. 2017).

2.5. Biochemical parameters

2.5.1. Determination of Chlorophyll and Proline Contents

The total chlorophyll content was estimated in pigeonpea leaves after 45 dpi, as described previously (Srivastava et al. 2025). Similarly, the proline content in the infected and control leaf tissues was estimated after extracting proline with sulfosalicylic acid, reacting with ninhydrin, and measuring the absorbance at 520 nm (Forlani et al. 2020).

2.6. Disease Severity Index

The aggressiveness of the pathogenic *Fusarium* strains (Rj, Jh, VnS, JnS1, Fz, and ND) was evaluated in relation to disease development (yellowing/wilting of pigeon pea plants) in both wilt-sensitive and wilt-resistant varieties by calculating the disease severity index. Plants were classified into severity classes according to the grading system described by (Willoquet et al. 2023). The disease severity index (DSI) was determined using the following formula:

$$\Sigma \text{DSI (\%)} = (\text{class} \times \text{score of rating class}) / [(\text{total number of observations}) \times (\text{maximal disease index})] \times 100.$$

2.7. Plant characteristics

The effect of *Fusarium* infestation on the growth of pigeon pea plants was determined in terms of plant height and root length (cm). of branches, no. of pods and seeds and seed weight (g) for the greenhouse-grown plants after 45 dpi.

2.8. Statistical analysis

The significance and analysis of variance between different variables for the results obtained from the *in vitro* and *in vivo* experiments were calculated, and the standard errors for the arithmetic means are mentioned throughout the manuscript. The pathogenic effects of *Fusarium* strains on pigeon peas were determined using Tukey's test of significance using IBM SPSS 20.0 software.

3. Results

3.1. Characterization of pathogenic strains responsible for *Fusarium* wilt in pigeonpea

Pigeon pea plants showing typical symptoms of *Fusarium* wilt, such as yellowing leaves, stem discoloration, and blackened inner tissue, were collected from different areas in Uttar Pradesh (Fig. S1A). Samples from 16 main pigeon pea sites were used to isolate the *Fusarium* strains that caused the disease. A total of 97 possible strains, identified based on their morphological similarities to *Fusarium* spp., were isolated (Fig. S1B).

3.1.1. Pectate lyase & Cellulase as pathogenicity traits

To determine the level of pathogenicity among the isolated 97 *Fusarium* strains, the cell wall-degrading enzymes pectate and cellulase were quantitatively measured in the fungal biomass (Fig. S2). There was

variability among the 97 isolates, and five strains demonstrated high levels of enzyme activity. Wherein, pectate lyase activity were as follows: Rj 4.77 ± 0.027 or (477%) > Fz 2.65 ± 0.036 or (265%) > Jh 2.64 ± 0.036 (264%) > JnS1 2.61 ± 0.017 (261%) > VnS 2.58 ± 0.058 (258%). Likewise, in cellulase activity were recorded as: Rj 27.86 ± 0.245 (278%) > JnS1 23.8 ± 0.20 (238%) > Fz 23.8 ± 0.20 (238%) > Jh 23.7 ± 0.23 (237%) > VnS 23.6 ± 0.29 (236%) (Fig. 1A and B).

3.1.2. *In vitro* plate assay for pathogenicity in pigeonpea

To discern the virulence of the five selected *Fusarium* strains, a comparative study was conducted between susceptible (IPAC-79) and resistant (BAHAR) pigeon pea varieties. The strain Rj (*Fusarium nygamai*) exhibited the highest level of infestation in pigeonpeas and demonstrated the highest germination inhibition rate in both the wilt-sensitive and wilt-resistant varieties. In contrast, the strains JnS1 VnS Jh and Fz showed a lower inhibition rate towards the wilt-sensitive variety compared to the wilt-resistant variety (Fig. 1D).

3.2. Identification of *Fusarium* isolates

3.2.1. Micromorphological assesment

JnS1 exhibited long, branched, septate hyphae with numerous small, single-celled, oval-to-kidney-shaped microconidia. Macroconidia formed fluffy, cottony, and aerial mycelia. The colony had a white centre with diffuse light pink-to-purplish pigmentation toward the outer margins. Rj microconidia were embedded within the mycelial mass, and macroconidia showed a dense, raised, white cottony centre surrounded by a dark pink-to-crimson purplish concentric ring. VnS showed abundant hyaline, oval macroconidia, and microconidia predominated, with a few slightly elongated structures, suggesting early or smaller macroconidia. Jh exhibited dense, compact, and velvety colonies with macroconidia. The colonies were mostly white with a yellowish or pale orange central tint. Thick-walled, dark, rounded chlamydospores serve as resting structures during stress. Fz showed uniform, dense, fluffy white growth, lacked pink, purple, or yellow pigments, and had heavily packed microconidia, indicating high sporulation efficiency (Fig. 1C).

3.2.2. Identification of *Fusarium* isolates pathogenic to pigeonpea

Fusarium isolates were successfully amplified (*ITS1/4* and *TEF 1 α*), and subsequent sequence analysis using NCBI BLAST confirmed that five isolates were members of the *Fusarium* genus. **Table 1** lists the GenBank accession numbers for the sequences submitted to the NCBI database. Fungal isolates with wilt symptoms were identified using morphological assessments and Multi-Locus Sequence Analysis (MLSA). PCR amplification of the Internal Transcribed Spacer (ITS) region and the Translation Elongation Factor 1-alpha (*TEF1- α*) gene resulted in fragments of approximately 550 and 600 bp, respectively. Initial BLASTn analysis of the ITS sequences led to the identification of all isolates as members of the *Fusarium* genus, with 99–100% homology. The isolate JnS1 clustered in a monophyletic group with the

Fusarium oxysporum reference strain (PV980321.1) with a 100% bootstrap support. The isolates Rj, VnS, Jh, and Fz clustered together and were stable, together with *Fusarium nygamai* (MT011009.1). The sequences were successfully deposited in the NCBI GenBank database with Accession No numbers PX845595, PX845596, PX845597, PX845598, and PX845599. The monophyly of *Fusarium* clades was confirmed using the outgroup *Rhizoctonia solani*. The high bootstrap values (generally > 90%) across the major nodes of the tree demonstrate the strong statistical reliability of this placement in the phylogenetic tree. (Fig. 1E).

3.2.3. Disease index

A greenhouse pot experiment was conducted using the same *Fusarium* strains with a sensitive variety (BAHAR), and the incidence of wilted plants under various treatments was significantly higher than that under the control. Specifically, JnS1 and Rj infestation caused a 309.60% and 439.12% increase in disease incidence, respectively. These findings demonstrate that all tested treatments substantially elevated the incidence of pigeon pea wilt compared to the untreated control, with Rj having the most pronounced effect (**Fig. S3A**). The total number of wilted plants in each treatment group was expressed as a percentage relative to that in the control group. The control group exhibited 0% wilting, whereas JnS1 showed a wilting rate of 200% relative to the control. RJ demonstrates the highest wilting at 300%, followed by Jh at 200%, with both VnS and Fz exhibiting 100% wilting (**Fig. S3B**).

3.3. Biochemical parameter:

3.3.1. Chlorophyll & Proline

Chlorophyll content in wilted pigeon pea plants decreased under all *Fusarium* infestation compared to controls in both sensitive and resistant varieties by 4.24 (mg/g) and 4.49 (mg/g) (Fig. 2A&2D). Roots infected with *Fusarium nygamai* (Rj) decreased the chlorophyll content by 70.37% and 68.82%, respectively, followed by Fz, VnS, JnS1, and Jh. Overall, all isolated *Fusarium* species significantly depleted chlorophyll. *Fusarium* infection increased proline content in both sensitive and resistant pigeonpea varieties. In the sensitive variety, the proline content increased from 31.57% in JnS1 to 53.84% in Rj, whereas in the resistant variety, it increased from 36.44% in Fz to 70.45% in Rj. Notably, JnS1 infestation induced a higher proline increase in the resistant variety (43.43%) than in the sensitive variety (31.57%). These results suggest that all *Fusarium* strains stimulated proline accumulation, with Rj having the strongest effect in both varieties (Fig. 2B and E).

3.4. *Fusarium* infestation impacts ROS profile in pigeonpea

The levels of stress markers in infected pigeon pea leaf tissues were monitored using MDA and H₂O₂. *Fusarium* infection significantly increased MDA and H₂O₂ levels in both sensitive and resistant pigeon pea varieties. In the sensitive variety, JnS1, RJ, VnS, Jh, and Fz infection caused an increase in MDA content in the order 65.18%>87.76%>69.62%>70.91%>73.86%, relative to the control. Similarly, in the resistant variety, JnS1, RJ, VnS, Jh, and Fz infection elevated MDA content by 49.37%, 75.27%, 50.37%,

50.45%, and 50.28%, respectively, compared to that of the control. The H₂O₂ content in the sensitive variety of pigeon pea increased in the following order: Rj (47.83%) > Jh (36.44%) > JnS1 (36.43%) > VnS (35.71%) > Fz (35.99%). In the resistant variety, the order was Rj (44.55%) > VnS (37.12%) > JnS1 (36.68%) > Fz (36.56%) > Jh (36.41%). These consistent increases indicated *Fusarium* infection-induced oxidative stress (Fig. 2C and F).

3.4.1. Physiological Parameters

The sensitive varieties exhibited reductions in shoot length of 14.81% (JnS1), 13.73% (Rj), 15.44% (VnS), 16.69% (Jh), and 14.19% (Fz) compared with the control. In contrast, the resistant varieties showed greater reductions of 17.33% (JnS1), 15.26% (Rj), 17.97% (VnS), 17.02% (Jh), and 17.55% (Fz.) in shoot length. Regarding root length, the sensitive variety demonstrated reductions of 14.76% (JnS1), 12.86% (Rj), 15.24% (VnS), 16.19% (Jh), and 15.71% (Fz), whereas the resistant variety exhibited reductions of 19.64% (JnS1), 12.58% (Rj), 12.27% (VnS), 19.03% (Jh), and 15.34% (Fz). The number of branches decreased by 16.75% (JnS1), 14.42% (Rj), 16.28% (VnS), 16.75% (Jh), and 18.61% (Fz) in the sensitive variety, while the resistant variety showed reductions of 15.90% (JnS1), 11.66% (Rj), 17.31% (VnS), 18.37% (Jh), and 16.96% (Fz). Dry weight was reduced by 16.39% (JnS1), 12.71% (Rj), 17.12% (VnS), 16.63% (Jh), and 16.63% (Fz) in the sensitive variety, compared to reductions of 16.95% (JnS1), 11.71% (Rj), 17.36% (VnS), 16.31% (Jh), and 16.31% (Fz) in the resistant variety (Fig. 5A-E) & (Fig. 5F-J).

3.5. Impact of *Fusarium* species complex in pigeonpea wilting

In evaluating the impact of *Fusarium* species on pigeon peas, RJ (*Fusarium nygamai*) was found to be the most prevalent and resilient strain, demonstrating significant virulence even under adverse conditions. However, *Fusarium udum* has been identified as the primary pathogen responsible for wilting in pigeon peas. To assess the pathogenicity of both *Fusarium udum* (ND) and *Fusarium nygamai* (RJ), the *Fusarium udum* (ND) strain was sourced from the ICAR NEW DELHI repository, and its effects on pigeon pea seedlings were evaluated. Additionally, the infection of *Fusarium nygamai* (RJ) was studied independently and in combination with *Fusarium udum* as a *Fusarium* sp. complex (RJ + ND) to determine their collective impact on wilting in pigeon pea plants.

3.6. Impact of *Fusarium* species complex on Disease Index in pigeonpea

Subsequently, the disease severity index was determined for the *Fusarium* spp. complex. Pigeonpea wilt showed an increased disease incidence under all treatments (RJ, ND, and RJ + ND) compared to the control in both susceptible and resistant varieties. In the susceptible variety, the disease index increased by 306% for RJ + ND at 21 dpi (Fig. 4A). The resistant variety showed an increase in disease incidence at 7 days post-inoculation (dpi) (Fig. 4B). These results indicate that the *Fusarium* complex exacerbates wilt severity, highlighting the need for further investigation of its biological and agronomic effects.

Simultaneously, the sensitive variety of *Fusarium* species complex (RJ + ND) showed the highest wilt incidence, beginning with three wilted plants at 15 dpi, increasing to four at 21 dpi, and reaching five at 45 days post-inoculation (dpi) (Fig. 4C). The control group of the wilt-resistant variety had no wilted plants. Rj increased from 1 to 2 wilted plants at 45 dpi, and Nd increased from 1 to 2 at 30 and 45 dpi. ND + RJ in the resistant variety started at two wilted plants at 15dpi, rising to three from 21 dpi onwards (Fig. 4D). Overall, wilt-sensitive plants exhibited higher wilt numbers across treatments and time points, with the *Fusarium* complex (RJ + ND) consistently causing the greatest wilt incidence, suggesting a potential additive effect over time.

3.7. Biochemical & Physiological parameters

In the wilt-sensitive variety (BAHAR), a comparison with the control revealed a reduction in total chlorophyll of 75.44% in the RJ treatment, 33.58% in the ND treatment, and 65.41% in the RJ + ND treatment. The proline content decreased by 37.53% in RJ and 60.61% in ND, whereas it increased by 149.2% in RJ + ND. In the wilt-resistant variety (IPAC-79), total chlorophyll decreased by 67.63% in the RJ treatment, 49.86% in the Nd treatment, and 56.29% in the RJ + ND treatment compared to the control group. The proline content decreased by 69.73% in RJ but increased by 10.06% in ND and 41.73% in Rj + ND groups. In the wilt-sensitive variety (BAHAR), shoot length was reduced by 40.77% in the RJ treatment, 38.34% in the ND treatment, and 41.36% in the RJ + ND treatment. Root length decreased by 38.17%, 42.43%, and 36.84% in the RJ, ND, and RJ + ND groups, respectively. The number of branches decreased by 33.33%, 11.2%, and 31.47% in the RJ, ND, and RJ + ND groups, respectively. Dry biomass was reduced by 24.04% in the Rj, 26.91% in the ND, and 18.22% in the RJ + ND treatments. In the wilt-resistant variety (IPAC-79), shoot length was reduced by 42.13%, 39.95%, and 42.13% in the RJ, ND, and RJ + ND treatments, respectively. Root length decreased by 26.03%, 34.21%, and 42.43% in the RJ, ND, and RJ + ND groups, respectively. The number of branches decreased by 28.57%, 14.29%, and 26.29% in the RJ, ND, and RJ + ND groups, respectively. Dry biomass decreased by 16.67% in RJ, 20.00% in ND, and 21.20% in RJ + ND (Table S2).

3.8. Effect of *Fusarium* wilt complex on morpho-physiological parameters of pigeonpea root Anatomy

Fusarium infestation induces changes in root morphology in sensitive plant varieties. Browning observed in the stem pith is indicative of necrosis and tissue death. The presence of collapsed or fragmented pith suggests degradation of the parenchymatous tissue, and obstruction of the xylem vessels results in impaired water transport. The presence of hyphal strands is indicative of *Fusarium nygamai* and *Fusarium udum* infections. The red arrows in **ND** highlight areas of tissue damage and pathogen activity (Fig. 6C). A correlation was observed between pith damage and wilting symptoms. The progression of damage from Control to Rj, Nd, and Rj + Nd indicated an increase in pathogen severity (Fig. 6C).

3.9. *Fusarium* complex infestation impacts ROS profile in pigeonpea

The MDA values in wilt-sensitive varieties increased by 95.5% under Rj treatment, 40.5% under ND treatment, and 36.9% under combined *F. udum* and *F. nygamai* (ND + RJ) treatments relative to control. In contrast, the MDA levels of wilt-resistant varieties increased by 30.8%, 20.0%, and 22.4% under the RJ, ND, and *F. udum* and *F. nygamai* (ND + RJ) treatments, respectively, compared to the control. These findings demonstrated a markedly greater percentage increase in MDA levels in wilt-sensitive varieties across all treatments compared to that in the wilt-resistant varieties (Fig. 3C and G).

The H₂O₂ content significantly increased in both wilt-sensitive and wilt-resistant varieties compared to their respective controls. In the sensitive variety, H₂O₂ levels increased by 23.6%, 16.5%, and 32.3%. Similarly, the numbers of resistant varieties increased by 33.4%, 32.9%, and 53.3%, respectively. These results indicate a more pronounced relative accumulation of H₂O₂ in the resistant variety under treatment conditions, particularly with the combined *F. udum* and *F. nygamai* (ND + RJ treatment) (Fig. 3D and H).

4. Discussion

Pigeonpea is a key source of plant-based proteins, dietary fibre, essential vitamins, and phytochemicals for millions of people (Susmitha et al. 2022). Globally, approximately 4.49 million tonnes of pigeonpeas are produced annually, with India contributing nearly 63% of the total production and 72% of the global cultivation area (Jha et al. 2024). Pigeonpea production faces major losses due to *Fusarium* wilt, a soil-borne disease (Kannaiyan et al. 1984; Rangaswamy et al. 2016). In India, *Fusarium* wilt in pigeonpeas leads to an annual production loss of up to 0.1 million tonnes (Rangaswamy et al. 2016). *Fusarium* spp. can remain in the soil for many years and spread through infected seeds, soil, and plant debris, making them difficult to control (Nikitin et al. 2023). In this study, we assessed the occurrence of *Fusarium* wilt disease in pigeon peas in Uttar Pradesh. *Fusarium* isolates were characterised from multiple locations using an integrative approach of morphological and molecular characterisation combined with multi-locus phylogenetic analyses.

The isolation of 97 *Fusarium* strains from symptomatic pigeonpea plants in Uttar Pradesh and the identification of five highly pathogenic strains with high pectate lyase and cellulase activity indicate the importance of cell wall-degrading enzymes in the pathogenicity of *Fusarium*. The identified strain was the causal agent *F. nygamai*. The presence of *Fusarium nygamai*, which is the most active and virulent, confirms previous results that increased production of pectinolytic and cellulolytic enzymes is correlated with increased aggressiveness of the fungus in different host plants (Perincherry et al. 2021). Based on these results, it was concluded that enzymatic profiling is a valuable tool for predicting the pathogenic potential of *Fusarium* isolates of pigeonpeas. High virulence was corroborated by greenhouse experiments, where disease incidence was significantly higher in susceptible pigeonpea varieties, especially with Rj and JnS1. The more severe wilt symptoms that occur in this region are due to the presence of Rj, and hence its dominance as a pathogen.

(Mehta et al. 2013) studied the morphological and physiological variations of *F. udum* isolates, such as mycelial growth pattern, colour variation, and pigmentation of the substrates. Mycelial growth was sparse to abundant, fluffy to suppressed or scanty fibrous, and yellow/white to greyish purple as the cultures progressed in age (2 to 7 days after inoculation). The isolates exhibited different colony morphologies and conidial structures, consistent with the taxonomic descriptions of *Fusarium* species complexes (Leslie et al. 2006). Molecular characterisation of the isolates was performed by DNA sequence analysis of the *ITS* and *TEF1- α* loci, which showed that JnS1 clustered with *Fusarium oxysporum* and the others with *Fusarium nygamai*, confirming the quality of multi-locus sequence typing (MLSA) in *Fusarium* taxonomy (O'Donnell K et al. 2013). The outgroup of *Rhizoctonia solani* further supported the phylogenetic placement, and monophyly was confirmed with high bootstrap support values. These results were similar to those of recent studies (Singh et al. 2020) which showed the importance of using complementary morphological and molecular methods to diagnose *Fusarium* species in plant pathology.

The different pathogenicity observed between susceptible (IPAC-79) and resistant (BAHAR) varieties and the highest level of germination inhibition caused by *F. nygamai* (Rj) fit previous reports, highlighting the host genotype effect on the virulence expression of *Fusarium* (Fatima et al. 2024; Huang X et al. 2019). A reduction in shoot and root lengths, branch numbers, and dry weight of both susceptible and resistant varieties validated the negative effect of *Fusarium* infection on plant growth and development. The overall higher reductions for the resistant variety could be a cost of defense activation and growth, as has been reported in other pathosystems (Huot et al. 2014). These physiological defects are probably a result of vascular obstruction and disruption of nutrient transport resulting from *Fusarium* colonisation, which is supported by anatomical observations. In other studies, on wheat, maize, chickpea, cabbage, tomato, banana, cowpea, pea, and barley pathosystems, there was a consistent contrast between resistant and sensitive genotypes with regard to inhibition of seedling performance, increased colonisation, or increased pathogen-responsive damage (Tran et al. 2021; Ping X et al. 2024; Sable et al. 2025). The variation in wilting percentage among the isolates may be due to variability among the pathogens and host-pathogen interactions and should be considered when selecting breeding lines for resistance (Maymon M et al. 2021). There was a substantial decrease in chlorophyll content in all *Fusarium* infections which is a common symptom of vascular wilt disease (Daniel I et al. 1949). The highest levels of chlorophyll depletion were produced by the more virulent *Fusarium nygamai*. The accumulation of proline, particularly in resistant cultivars, supports the hypothesis that it acts as an osmoprotectant and stress reliever during infection, consistent with its involvement in stress-induced proline accumulation in other host–pathogen systems, which has been reported to be associated with greater resistance to biotic stress (Kishor et al. 2015). The different proline responses of susceptible and resistant varieties indicate that host defense mechanisms are complex. The malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels in infected plants indicated that *Fusarium* infection led to oxidative stress, as manifested by lipid peroxidation and accumulation of reactive oxygen species (ROS). The greater increase in MDA for the susceptible varieties than the resistant varieties indicates that the membranes are more damaged and stressed in the susceptible varieties, as reported in an earlier study

on *Fusarium* wilt of legumes (Tripathi et al. 2020). Interestingly, the accumulation of H_2O_2 was relatively higher in resistant varieties, which could indicate a controlled burst of ROS in defense signalling responses (Mittler et al. 2017). (Pollard et al. 2018) reported similar enzyme-type defence mechanisms in legumes where the resistant accessions of chickpea, pea and other species exhibited high concentrations of chitinases, glucanases, proteases and lignin-biosynthetic enzymes in their roots, with these enzymes being correlated with inhibition of spore germination, hyphal growth and xylem invasion by *Fusarium oxysporum*. The results highlight the dual function of ROS in plant-pathogen interactions as pathogen damaging agents and signalling molecules.

Plants inoculated with the *Fusarium* complex containing *F. udum* and *F. nygamai* (Rj + Nd) exhibited increased disease severity compared to plants inoculated with either pathogen alone, indicating an additive or synergistic effect of pathogen complexes, which has been reported in other pathogen-host systems and crop-fungal diseases (Jones et al. 2006). In Israel, (Maymon et al. 2021) isolated four *Fusarium* species complexes from jojoba wilt samples: *F. oxysporum*, *F. fujikuroi*, *F. hostae*, and *F. solani* (Deepak Reddy B et al. 2024). In the present study, combined inoculation with *Fusarium nygamai* and *Fusarium udum* (RJ + ND) caused the most severe wilt symptoms and incidence, implying a possible synergistic action that worsens the disease effect. The levels of oxidative stress markers, MDA and H_2O_2 , showed the same trend across the treatments, confirming that reactive oxygen species (ROS) play a role in cell damage caused by *Fusarium*. The significant difference in the increase in MDA levels in resistant and sensitive varieties in complex infections by *F. udum* and *F. nygamai* shows that susceptible varieties are more susceptible to lipid peroxidation and oxidative damage under these infections. However, the higher levels of H_2O_2 in resistant varieties, particularly in the combination treatment of *F. udum* and *F. nygamai* (RJ + ND), indicate increased ROS-mediated defense signalling. This pattern is consistent with recent evidence of the role of controlled ROS production in initiating systemic acquired resistance and programmed cell death for pathogen control (Foyer et al. 2013). Pathogenicity assays confirmed that the *Fusarium* species caused typical *Fusarium* wilt symptoms in pigeonpeas.

These findings underscore the importance of adopting an integrated management approach to control multiple *Fusarium* species for effective pigeonpea wilt management. Combined infection by *Fusarium* significantly decreased chlorophyll and growth parameters and produced variable responses of proline, suggesting increased stress levels. The differential accumulation of proline between sensitive and resistant varieties in response to complex infections is likely to indicate metabolic changes and defense strategies observed in other active host-pathogen interactions (Zhou et al. 2019). Biochemical and physiological disturbances lead to severe wilting symptoms. The destructive effect of *Fusarium* spp. on the vascular tissues of infected roots is confirmed by the presence of histopathological changes, including pith browning, degradation of the parenchyma, and obstruction of the xylem vessels, which result in impaired water transport and wilting. There is evidence of active fungal colonisation, with the presence of hyphal strands in damaged tissues. The anatomical changes are similar to those reported as a typical *Fusarium* wilt disease of legumes and support the correlation between structural damage and disease symptomology (Agrios G. 2008). Numerous studies have shown that *Fusarium nygamai* is

the first novel strain associated with basal rot in onion and beans (Rajakumara et al. 2024). *Fusarium nygamai* has been reported to cause crown rot in wheat in Kazakhstan and China (Zhang et al. 2023; Vega Gutiérrez et al. 2022; Kharipzhanova et al. 2025). *Fusarium udum* infecting pigeonpeas was isolated from seven states of India, and it was reported as the primary pathogen causing pigeonpea wilt in the Bundelkhand region of Uttar Pradesh, with a disease incidence ranging from 3.25% to 49.00% across four districts. Chitrakoot, Banda, Hamirpur, and Mahoba (Mehta et al. 2013; Singh et al. 2023). Study by (Mishra et al. 2021) reported that wild pigeonpea, *Cajanus scarabaeoides*, wilt disease caused by *F. equiseti*, *F. luffae*, *F. compactum*, *F. nygamai*, *F. citri*, *F. ipomoeae*, and *F. curvatum* represent new records on producing lesions on detached cherry leaves of sweet cherry in China (Zhou et al. 2022). The present study identified *Fusarium nygamai* as a prevalent co-infecting species, highlighting the complexity of the aetiology of *Fusarium* wilt in pigeon peas. This is the first report of *F. nygamai* causing *Fusarium* wilt in pigeonpea. These findings expand the known host range of pathogens and provide important baseline information for diagnosing diseases in *Fusarium* species that cause crop wilt. These species are commonly found in areas of Uttar Pradesh where pigeonpea wilt disease occurs.

5. Conclusion

In conclusion, *Fusarium nyagami* was isolated and identified as the causative agent of *Fusarium* wilt in pigeon peas in Uttar Pradesh, marking the first report of this pathogen in this host. These findings expand the known host range of fungi and reveal previously unrecognised pathogen diversity associated with pigeonpeas. These results provide essential baseline information for accurately determining the pathogenicity of *Fusarium* species and emphasise the importance of pathogen monitoring for the effective management of *Fusarium* wilt in plant systems.

Declarations

Author Contribution

All authors contributed to the study conception, design, and manuscript preparation. Conceptualisation was performed by SuS. Investigation and experimental execution were carried out by RF, PK, and AA. Formal analysis and in vivo studies were conducted by PK and AA. The first draft of the manuscript was written by RF. Critical revision and editing of the manuscript were performed by RF, Rk and AD. All authors read and approved the final manuscript.

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Figures

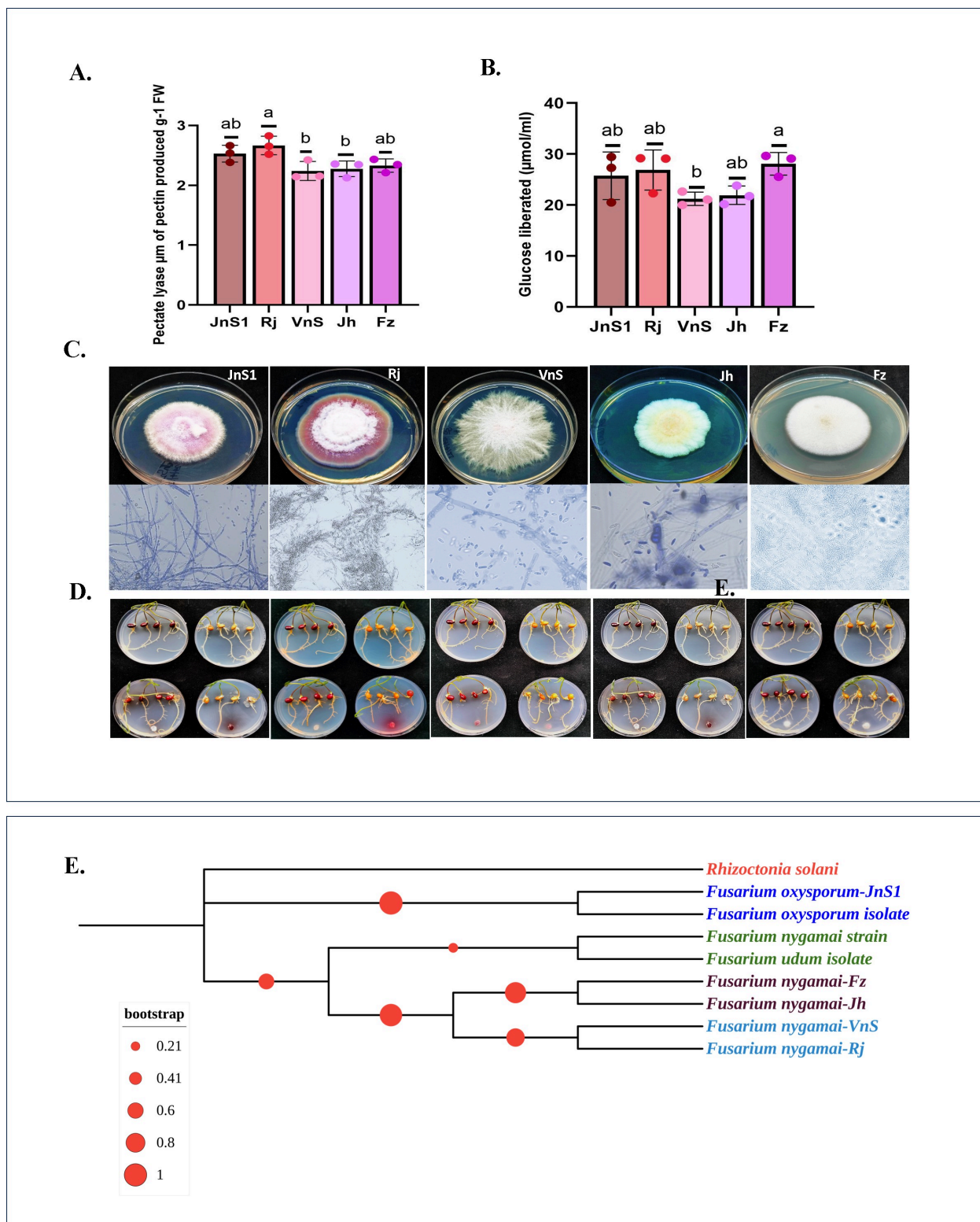


Figure 1

(A, B) Characterisation of *Fusarium* isolates for pectinase and cellulase activity. (C) *Fusarium* colonies on PDA agar and their conidial morphology. (D) Pathogenicity assessment of the *Fusarium* isolates. (E) Phylogenetic analysis based on concatenated *ITS* and *TEF-α* sequences with 1000 bootstrap replicates; *Rhizoctonia solani* was used as the outgroup.

****Note:** *Fusarium nygamai* is denoted as RJ, VnS, Jh, Fz; *Fusarium oxysporum* is denoted as JnS1

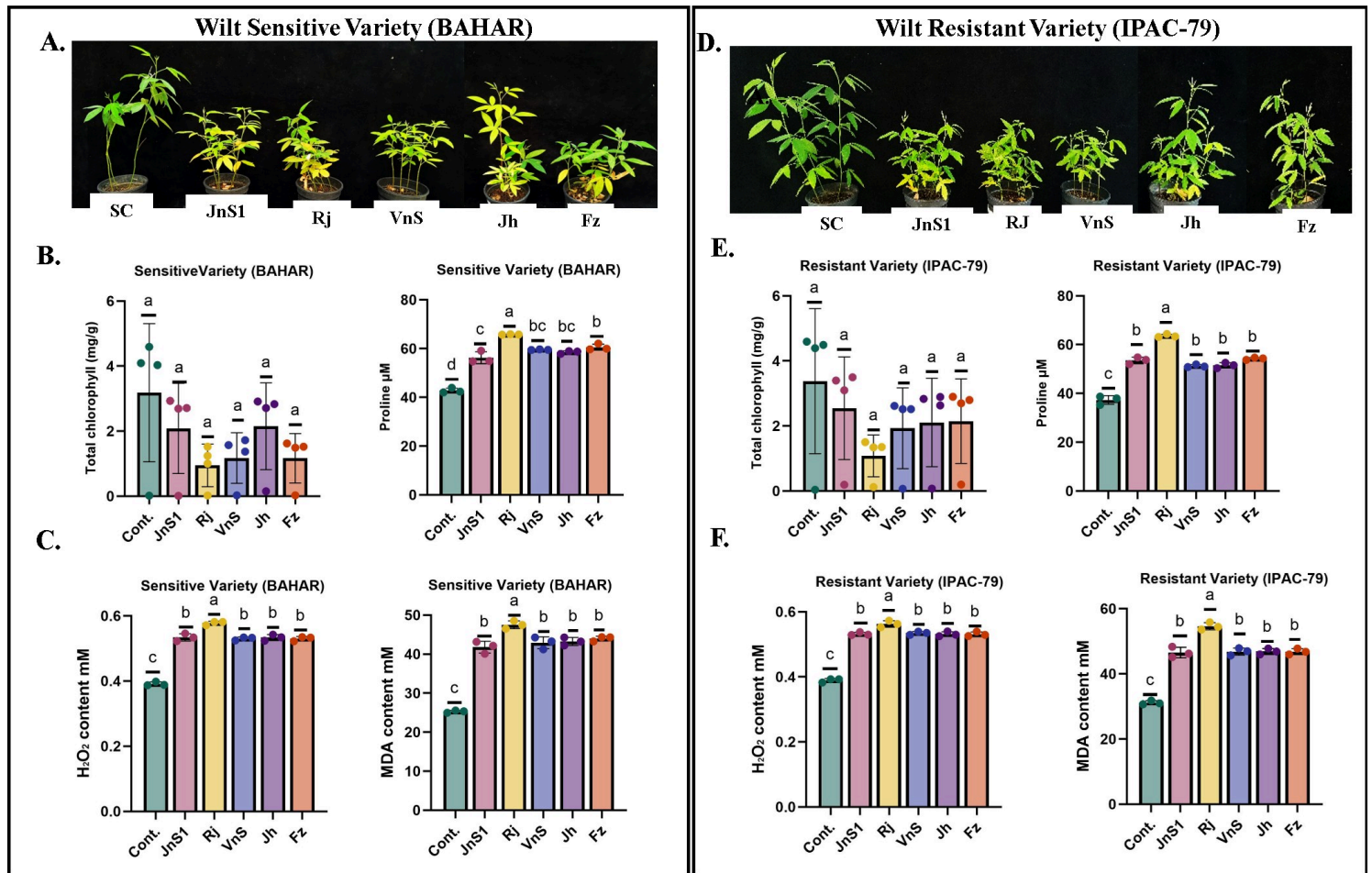


Figure 2

Impact of *Fusarium* infection on the physiological parameters of pigeonpea varieties: (A, D) Chlorophyll and proline contents in wilt-sensitive and wilt-resistant varieties, respectively; (B, E) malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) contents; (C, F) additional physiological measurements.

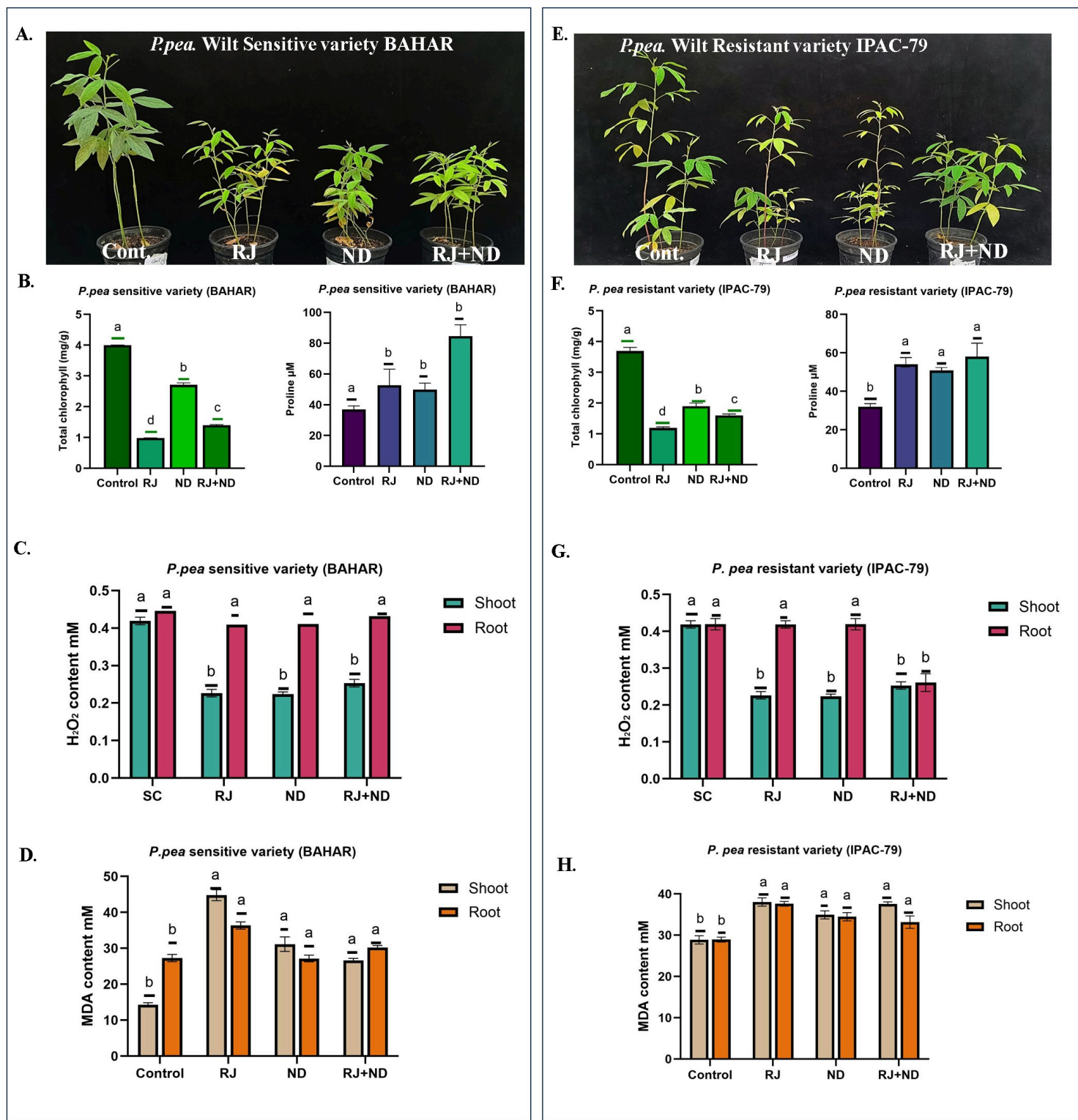


Figure 3

Modulation of physiological and reactive oxygen species (ROS) profiles in pigeon peas during *Fusarium* infection: (A, E) wilt-sensitive (BAHAR) and wilt-resistant (IPAC-79) varieties; (B, F) total chlorophyll and proline levels; (C, G) H₂O₂ activity; (D, H) MDA activity.

***Note: Fusarium udum is denoted as ND, Fusarium complex is denoted as (RJ+ND)*

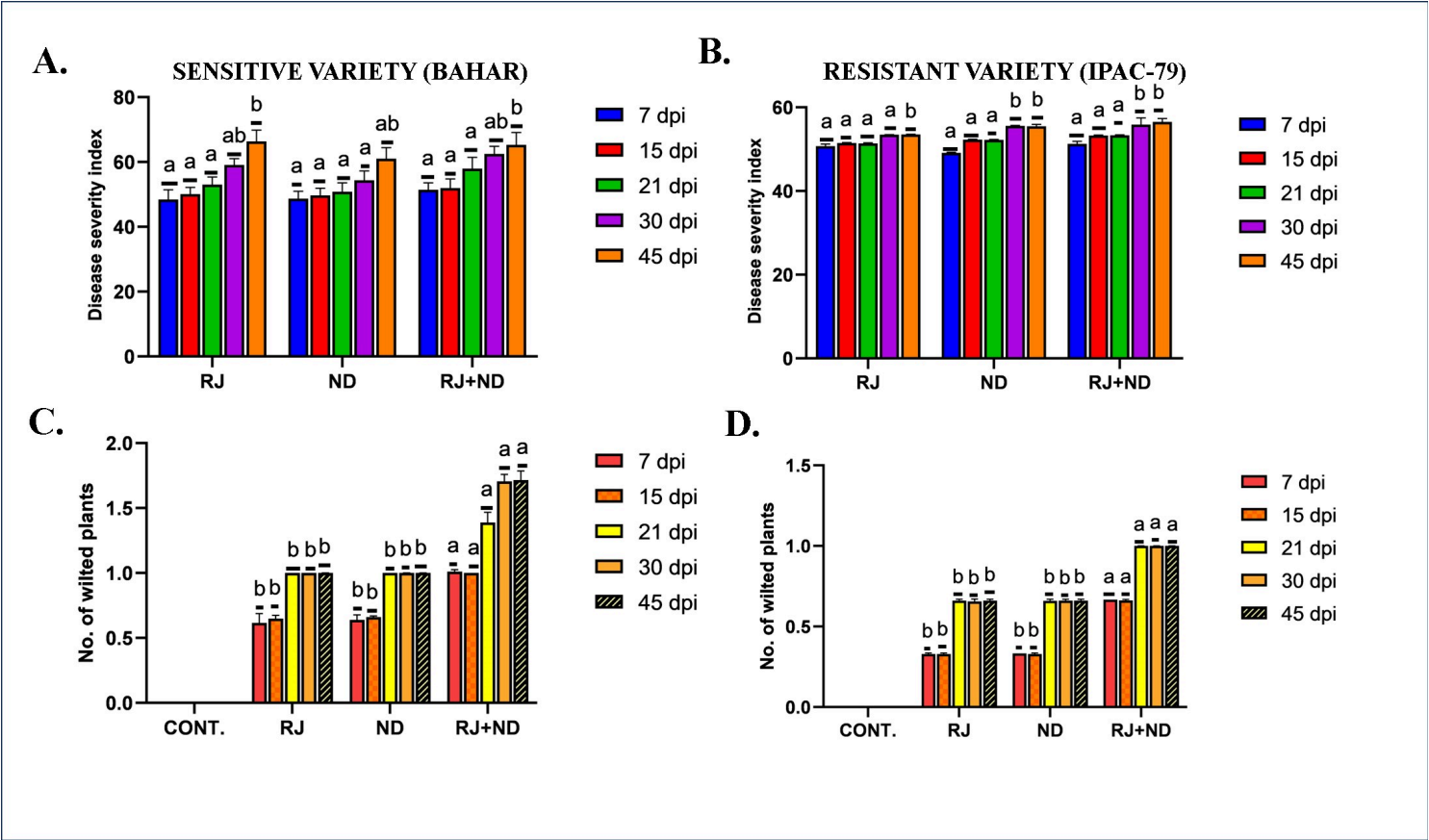


Figure 4

Estimation of *Fusarium* wilt progression in sensitive (BAHAR) and resistant (IPAC-79) pigeonpea varieties based on: (A, B) disease severity index; (C, D) number of wilted plants at 7, 15, 21, 30, and 45-days post-inoculation (DPI).

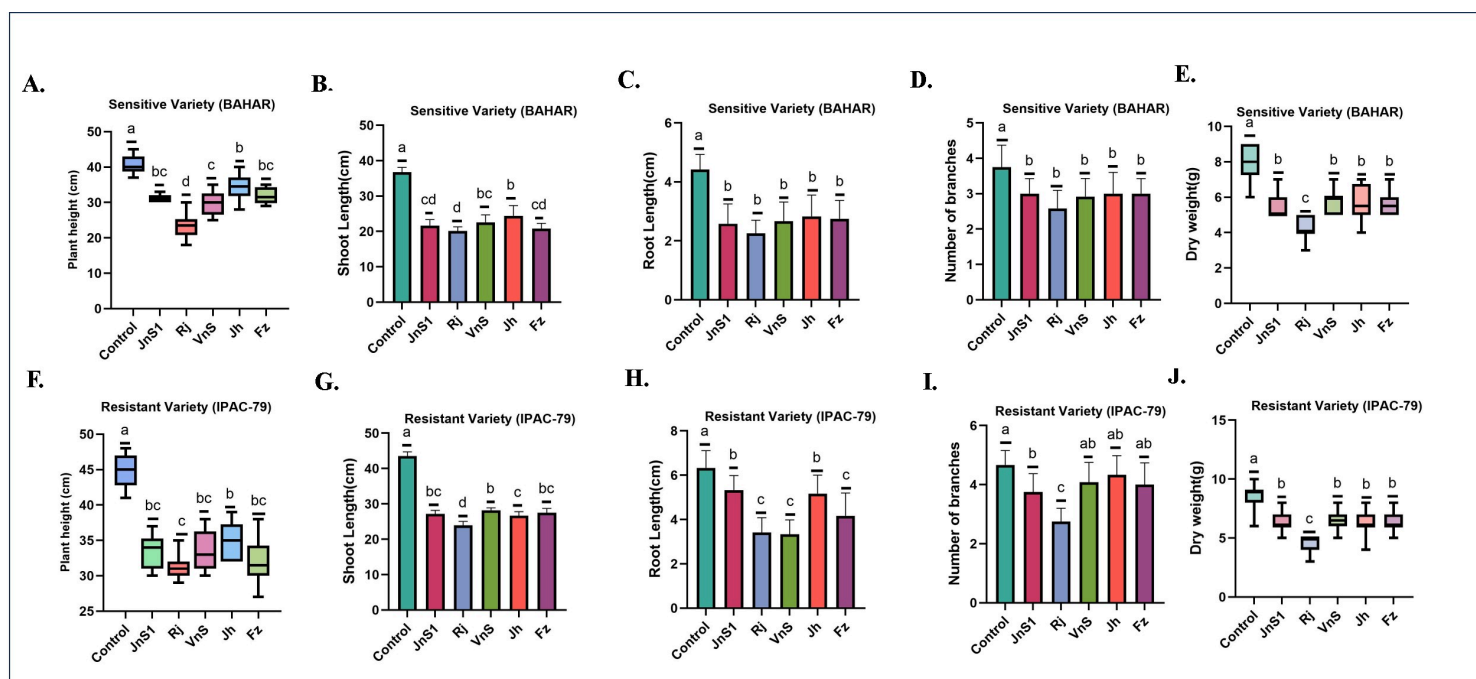


Figure 5

Effects of *Fusarium* infection on growth and physiological parameters of pigeonpea plants under microplot conditions.

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Figure 6

Effects of *Fusarium* infestation on the morphology of pigeonpea plants: (A) wilt-sensitive and (B) wilt-resistant varieties; (C) cotton blue histochemical staining of pigeonpea roots at 30 DPI, with arrows indicating root cortex damage resulting from *Fusarium* hyphal penetration.

Supplementary Files

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