



RESEARCH ARTICLE

Morphological and molecular diversity of *Alternaria porri* isolates causing purple blotch disease in small onion across Tamil Nadu

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Abstract

Purple blotch caused by *Alternaria porri* is one of the most destructive diseases limiting small onion (*Allium cepa* L.) production in Tamil Nadu. To understand the prevalence and variability of the pathogen, a comprehensive survey was conducted during 2024 across major onion-growing regions of the state. Disease severity varied considerably among locations, with percent disease index (PDI) values ranging from 9.80–76.80 %, indicating the widespread occurrence of the disease under diverse agro-climatic conditions. A total of 31 isolates of *A. porri* were recovered from infected onion samples and characterised using cultural, morphological and molecular approaches. The isolates exhibited marked variability in colony characteristics, sporulation, conidial morphology and virulence, suggesting the existence of diverse pathogen populations within the state. Pathogenicity studies identified several highly virulent isolates capable of causing severe disease symptoms. Molecular characterisation based on internal transcribed spacer (ITS) sequencing confirmed the identity of the isolates, showing 90–100 % similarity with reference *A. porri* sequences available in the National Center for Biotechnology Information (NCBI) database. Species identity was further validated using Alt a1 gene-specific primers. Phylogenetic analysis revealed moderate genetic diversity among the isolates and grouped them into distinct clusters, reflecting their evolutionary relationships. The study highlights the substantial spatial, morphological and molecular variability present in *A. porri* populations associated with onion purple blotch in Tamil Nadu and provides valuable baseline information for disease surveillance, resistance screening and the development of effective management strategies.

Keywords: *Alternaria porri*; morphological characterisation; phylogenetic analysis; purple blotch; small onion

Introduction

Onion (*Allium cepa* L.) is an economically important vegetable crop cultivated for its culinary, nutritional and commercial value. The crop is adaptable to a broad range of agro-climatic conditions and contributes significantly to food security and international trade in many countries. India ranks as the second-largest producer of onions globally, playing a crucial role in both domestic consumption and export markets (1). In recent years, onion cultivation in India has expanded markedly in terms of both cultivated area and total production, reaching approximately 30.77 million tonnes in 2024–25, thereby underscoring its growing importance within the horticultural economy (2).

Within the country, onions are cultivated across multiple seasons under diverse production systems, with major contributions from states such as Maharashtra, Madhya Pradesh, Karnataka, Gujarat and Rajasthan. This crop occupies a prominent position in the horticulture sector, consistently contributing more than 30 million tonnes to the national agricultural output in recent years (3). Despite periodic fluctuations driven by climatic variability, onion production has remained relatively stable, generally exceeding 30 million tonnes annually and continuing to serve as a key component of the agricultural economy (4).

In southern India, Tamil Nadu represents a distinct production system characterised predominantly by the cultivation of small onions (shallot type), in contrast to the large bulb onions cultivated in the northern regions. Small onions are widely preferred for their characteristic pungency, enhanced flavour and superior storage quality and they account for a substantial proportion of the onion-growing area in the state (5). The crop is extensively cultivated in districts such as Perambalur, Theni, Dindigul, Erode and Coimbatore under varied agro-climatic conditions in Tamil Nadu. Current estimates indicate that onion cultivation in Tamil Nadu spans approximately 45–50 thousand hectares (ha), with production ranging from 0.49–0.56 million tonnes and an average productivity of 10–12 t/ha (6). Consequently, small onion cultivation provides livelihood support to farmers and plays an important role in maintaining regional market supply chains.

Despite its economic importance, onion production is highly susceptible to various biotic stresses. Among these, purple blotch incited by *Alternaria porri* (Ellis) Ciferri is regarded as one of the most destructive foliar diseases affecting crops. The disease develops rapidly under warm and humid environmental conditions that favour pathogen growth and infection. The disease initially appears as small water-soaked spots that gradually enlarge over time into characteristic purplish spots with concentric rings surrounded by chlorotic halos, ultimately leading to leaf blight and reduced bulb development. Under conducive environmental conditions, yield losses may range from 30–80 % depending on the disease severity (7–9).

Although purple blotch is a major constraint in onion production, its incidence and severity vary across locations owing to differences in environmental conditions, agronomic practices and host susceptibility. Consequently, region-specific assessments of disease distribution are essential for developing effective management strategies. However, limited information is available regarding the distribution and severity of purple blotch disease in the major small onion-growing regions of Tamil Nadu. Several studies have reported morphological, pathogenic and molecular variability among *A. porri* isolates from onion-growing regions of Karnataka and other parts of India (8, 9). However, comparable information on the diversity and variability of *A. porri* populations associated with small onion cultivation in Tamil Nadu is limited. Therefore, detailed investigations on the occurrence, variability and molecular characterisation of the pathogen are essential for understanding its epidemiology and developing effective management strategies. This information is crucial for understanding pathogen variability and developing effective, region-specific disease management strategies. It was hypothesised that *A. porri* populations associated with purple blotch disease in the major onion-growing regions of Tamil Nadu exhibit significant variability in disease severity, cultural and morphological characteristics, pathogenicity and genetic diversity. Understanding such variability is essential for accurate pathogen identification and the development of effective disease management strategies. Therefore, the present investigation was carried out to evaluate (i) the occurrence and severity of purple blotch disease in the major small onion-growing areas of Tamil Nadu, (ii) isolate and characterise the associated pathogen based on cultural and morphological traits and (iii) confirm its identity using molecular approaches, including internal transcribed spacer (ITS) region amplification and Alt a 1 gene-specific markers.

Materials and Methods

Survey and documentation

A roving survey was carried out during the rabi season of 2024 in the major small onion-growing districts of Tamil Nadu, including Coimbatore, Perambalur, Theni, Salem, Erode and Dindigul, to assess the severity of purple blotch disease. In each district, 3–5 villages were selected based on the extent of small onion cultivation, accessibility of fields and the willingness of farmers to participate in the survey. Villages representing major onion-growing areas within each district were included to ensure adequate coverage of the disease situation. Within each village, three fields were surveyed and ten plants were randomly selected from each field for disease assessment. Consequently, a minimum of 30 plants per village were evaluated to ensure an adequate representation of disease incidence. Disease severity was recorded using a 0–5 rating scale, as described by Mayee and Datar (10), where 0 = no visible symptoms and 5 = complete drying of leaves. The percent disease index (PDI) was calculated using the formula (11).

$$\text{PDI} = \frac{\sum \text{Disease ratings} \times 100}{\text{Number of observations} \times \text{maximum disease grade}} \quad (\text{Eq.1})$$

Collection and isolation of the pathogen

Leaves showing characteristic purple blotch symptoms were collected from different surveyed locations and brought to the laboratory in sterile polyethylene bags for pathogen isolation. Small pieces of infected leaf tissue (3–5 mm) were excised and surface sterilised using 70 % ethanol for 30 s, followed by treatment with 1 % sodium hypochlorite solution for 1–2 min. The sterilised tissue bits were washed thrice with sterile distilled water to eliminate traces of disinfectants and dried gently using sterile blotting paper. Subsequently, the tissue segments were placed aseptically on potato dextrose agar (PDA) medium amended with streptomycin (50 mg L⁻¹) to minimise bacterial contamination. The plates were incubated at 25 ± 2 °C for 5–7 days under laboratory conditions. Developing fungal colonies were periodically observed and transferred to fresh PDA plates for purification. Pure cultures of the pathogen were established through single-spore and hyphal-tip isolation methods and maintained on PDA slants at 4 °C for further investigation following standard protocols (12).

Morphological identification of the pathogenic isolates

Purified fungal isolates were identified based on their cultural and microscopic characteristics. Cultural observations included colony colour, texture, margin and growth pattern on PDA medium. Microscopic examination was carried out under a compound microscope to study the conidial characteristics such as shape, size, septation and beak morphology. Temporary mounts were prepared using lactophenol cotton blue stain and conidial dimensions were measured using a calibrated ocular micrometer. The pathogen isolates were identified by comparing the observed characteristics with the standard taxonomic descriptions of *Alternaria* species (13, 14).

Molecular identification of the pathogenic isolates

Genomic DNA was isolated from actively growing fungal mycelia following the cetyltrimethylammonium bromide (CTAB) extraction method (15). The ITS region of the fungal isolates was amplified using the universal primer pair ITS1 (5'-TCCGTAGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (16). Polymerase

chain reaction (PCR) amplification was performed in a thermal cycler with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplified PCR products were separated on 1.2 % agarose gel containing ethidium bromide and visualised under UV light. A distinct amplicon of approximately 550–600 bp was obtained and subsequently purified for sequencing analysis. The generated ITS sequences were submitted to the NCBI GenBank database through the BankIt portal and accession numbers were assigned for all isolates to support pathogen identification. Phylogenetic analysis was performed using MEGA 11 software. Evolutionary distances were calculated using the Kimura 2-parameter model and the phylogenetic tree was constructed using the Neighbour-Joining method with 1000 bootstrap replications. Further confirmation at the species level was carried out using the *AsolDelta1* (5'-CGCATCTGCCCGTCT-3') and *AsolAlta1* (5'-GTTGGTGGCCTTGATGTTGAAG-3') (17). The thermal cycling conditions consisted of an initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 5 min. Amplification with these primers produced a characteristic band of nearly 118 bp, thereby confirming the pathogen as *A. porri*.

Pathogenicity assay of *Alternaria porri* isolates

Pathogenicity of the isolated *A. porri* isolates was confirmed by following Koch's postulates under pot culture conditions (18). Healthy onion seedlings were transplanted into 30 cm diameter earthen pots containing a sterilised soil: sand: farmyard manure mixture (2 : 1 : 1, v/v/v) and maintained under greenhouse conditions. Three plants were maintained per pot. After 20 days of transplantation, healthy onion leaves were inoculated with *A. porri* isolates by spraying a spore suspension (1×10^5 conidia mL⁻¹) prepared from 7-day-old cultures following the standard procedure (8). The spore concentration was determined using a haemocytometer and adjusted to the required concentration. In addition, mycelial discs (10 mm diameter) obtained from actively growing cultures were placed on onion leaves to facilitate infection. Both inoculation methods were applied simultaneously to ensure successful pathogen establishment and uniform disease development. The inoculated plants were maintained under high humidity conditions to promote symptom expression.

The experiment was conducted in a completely randomized design (CRD) with 3 replications for each isolate. After inoculation, the plants were covered with transparent polyethylene bags for 48 hr to maintain high humidity favourable for disease development. The inoculated plants were regularly observed for symptom appearance. Typical purple blotch symptoms such as necrotic lesions, chlorosis and leaf blighting developed within 5–7 days after inoculation, whereas no symptoms were observed in control plants sprayed with sterile distilled water. Disease severity was assessed using a 0–5 disease rating scale (10) and PDI was calculated using Eq. 1 (11).

Based on the PDI values recorded under pot culture conditions, the isolates were categorised into different virulence groups, namely highly virulent (> 60 % PDI), virulent (50–60 % PDI), moderately virulent (30–50 % PDI) and mildly virulent (< 30 % PDI), to assess pathogenic variability among the isolates.

To fulfill Koch's postulates, the pathogen was re-isolated from artificially infected leaves showing typical purple blotch symptoms and compared with the original culture based on morphological characteristics, thereby confirming the pathogenic nature of *A. porri* isolates. Disease severity was assessed using the 0–5 disease rating scale and expressed as PDI. The experiment was conducted in a CRD with 3 replications. The PDI data obtained from the pathogenicity test was subjected to analysis of variance (ANOVA) using AGRES statistical software and treatment means were compared at the 5 % level of significance.

Results

Survey on the incidence of purple blotch disease

A roving survey was conducted across major onion-growing districts of Tamil Nadu which revealed that purple blotch disease was widely prevalent, with considerable variation in severity among locations (Fig. 1). The PDI ranged from 9.80–76.80 %, indicating substantial spatial variability in disease severity. Among the surveyed districts, Theni recorded the highest disease severity, with PDI values ranging from 53.80–76.80 %. The maximum PDI (76.80 %) was recorded at TAP (THR)-20 (Theni), followed by TAP (UPP)-21 (67.30 %) and TAP (LGD)-22 (53.80 %). This was followed by the Madurai district, where the disease incidence ranged from 31.80–73.80 %. The highest PDI (73.80 %) was observed at MAP (ALA)-7, followed by MAP (KOT)-8 (68.30 %), whereas a comparatively lower incidence was recorded at MAP (MEL)-9 (31.80 %).

Moderate disease severity was observed in Dindigul (41.70–56.20 %) and Perambalur (27.50–53.40 %) districts. In Dindigul, the highest PDI was recorded at DAP (ODC)-23 (56.20 %), whereas in Perambalur, PAP (NKL)-16 (53.40 %) showed the highest disease severity. In contrast, a comparatively lower disease incidence was recorded in Coimbatore (21.50–28.60 %), where the highest PDI was observed at CAP (SAR)-6 (28.60 %) and the lowest at CAP (PER)-5 (21.50 %). Similarly, Erode (33.60–46.50 %) and Namakkal (31.60–33.80 %) showed moderate to low disease incidence. The lowest disease incidence was recorded in the Salem district, where the PDI ranged from 9.80 % to 16.50 %, with the minimum value observed at SAP (SAN)-29 (9.80 %), followed by SAP (ATR)-30 (10.30 %). The ANOVA revealed significant differences ($p \leq 0.05$) in disease severity among the surveyed locations. Based on Duncan's multiple range test (DMRT) grouping, Theni and Madurai recorded significantly higher PDI values, whereas Salem and Coimbatore exhibited significantly lower disease severity than the other districts. The variation in disease incidence observed across different locations could be associated with differences in environmental conditions such as temperature, relative humidity and local crop management practices, which play an important role in the development and spread of *A. porri*. Detailed location-wise disease severity values are presented in Table 1, while the spatial distribution of the disease severity is illustrated in Fig. 2 & Fig. 3. Based on district-wise mean PDI values, Theni (65.97 %) and Madurai (54.16 %) emerged as major hotspots for purple blotch disease, whereas Salem (12.20 %) and Coimbatore (24.70 %) recorded the lowest disease severity. These findings demonstrate substantial regional variability in purple blotch occurrence across Tamil Nadu.

Table 1. Incidence of purple blotch disease in onion across different districts of Tamil Nadu

District	Isolate code	Location	Latitude (N)	Longitude (E)	PDI (%)	Average PDI
Coimbatore	CAP (NAR)-2	Narasipuram	10.96	76.81	23.60 (29.10) ^{pq}	24.72
	CAP (THU)-3	Thudiyalur	11.08	76.94	24.30 (29.50) ^{pq}	
	CAP (THD)-4	Thondamuthur	10.98	76.84	25.50 (30.30) ^{op}	
	CAP (PER)-5	Perur	10.97	76.91	21.50 (27.60) ^{qr}	
	CAP (SAR)-6	Saravanampatti	11.07	77.00	28.60 (32.30) ^{no}	
Madurai	MAP (ALA)-7	Alanganallur	10.04	78.09	73.80 (59.20) ^b	54.16
	MAP (KOT)-8	Kottampatti	10.22	78.37	68.30 (55.70) ^c	
	MAP (MEL)-9	Melur	10.03	78.34	31.80 (34.30) ^{mn}	
	MAP (TWR)-10	Thirumangalam	9.82	77.98	49.20 (44.60) ^f	
	MAP (PAL)-11	Palamedu	10.10	78.11	47.70 (43.60) ^{fg}	
Perambalur	PAP (LAD)-12	Ladapuram	11.24	78.73	47.30 (43.40) ^{fg}	42.59
	PAP (MEL)-13	Melapuliyur	11.26	78.76	42.60 (40.70) ^{ij}	
	PAP (AMM)-14	Ammapalayam	11.23	78.85	39.50 (39.00) ^{jk}	
	PAP (SEN)-15	Senjeri	11.23	78.82	35.60 (36.70) ^{kl}	
	PAP (NKL)-16	Nakkaselam	11.16	78.72	53.40 (47.00) ^{de}	
	PAP (THR)-17	Thirupeyar	11.25	78.80	50.20 (45.10) ^{ef}	
	PAP (KUR)-18	Kurumbalur	11.23	78.80	44.60 (41.90) ^{hi}	
	PAP (PAL)-19	Palayam	11.23	78.88	27.50 (31.60) ^{no}	
Theni	TAP (THR)-20	Theni	10.00	77.47	76.80 (61.20) ^a	65.97
	TAP (UPP)-21	Iruppidam	9.93	77.47	67.30 (55.10) ^c	
	TAP (LGD)-22	Lalgudi	10.00	77.47	53.80 (47.30) ^{de}	
Dindigul	DAP (ODC)-23	Oddanchatram	10.48	77.75	56.20 (48.60) ^d	49.17
	DAP (NLT)-24	Nilakottai	10.16	77.85	49.60 (44.80) ^{ef}	
	DAP (NAT)-25	Natham	10.22	78.22	41.70 (40.20) ^{ij}	
Erode	EAP (PLR)-26	Perundurai	11.27	77.58	33.60 (35.50) ^{lm}	26.70
	EAP (SAT)-27	Sathyamangalam	11.50	77.24	46.50 (42.90) ^{gh}	
Salem	SAP (MET)-28	Mettur	11.78	77.80	16.50 (23.90) ^{rs}	12.20
	SAP (SAN)-29	Sankagiri	11.47	77.86	9.80 (18.30) ^t	
	SAP (ATR)-30	Attur	11.59	78.59	10.30 (18.70) st	
Namakkal	NAP (TRI)-31	Tiruchengode	11.37	77.89	33.80 (35.60) ^{lm}	32.70
	NAP (RKP)-32	Rasipuram	11.46	78.19	31.60 (34.20) ^{mn}	
CD (<i>P</i> = 0.05 %)			1.77			

PDI: Percent disease index.

Data in the parentheses are arcsine transformed values.

Means in a column followed by same superscript are not significantly different by DMRT at $p = 0.05$.

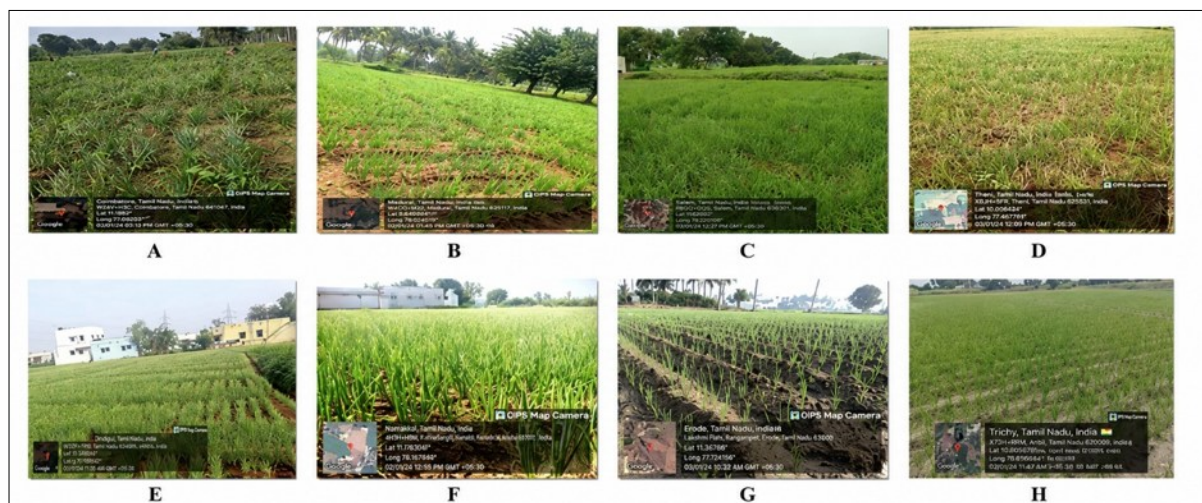


Fig. 1. Survey of major onion-growing areas of Tamil Nadu: (A) Coimbatore, (B) Madurai, (C) Salem, (D) Theni, (E) Dindigul, (F) Namakkal, (G) Erode and (H) Perambalur.

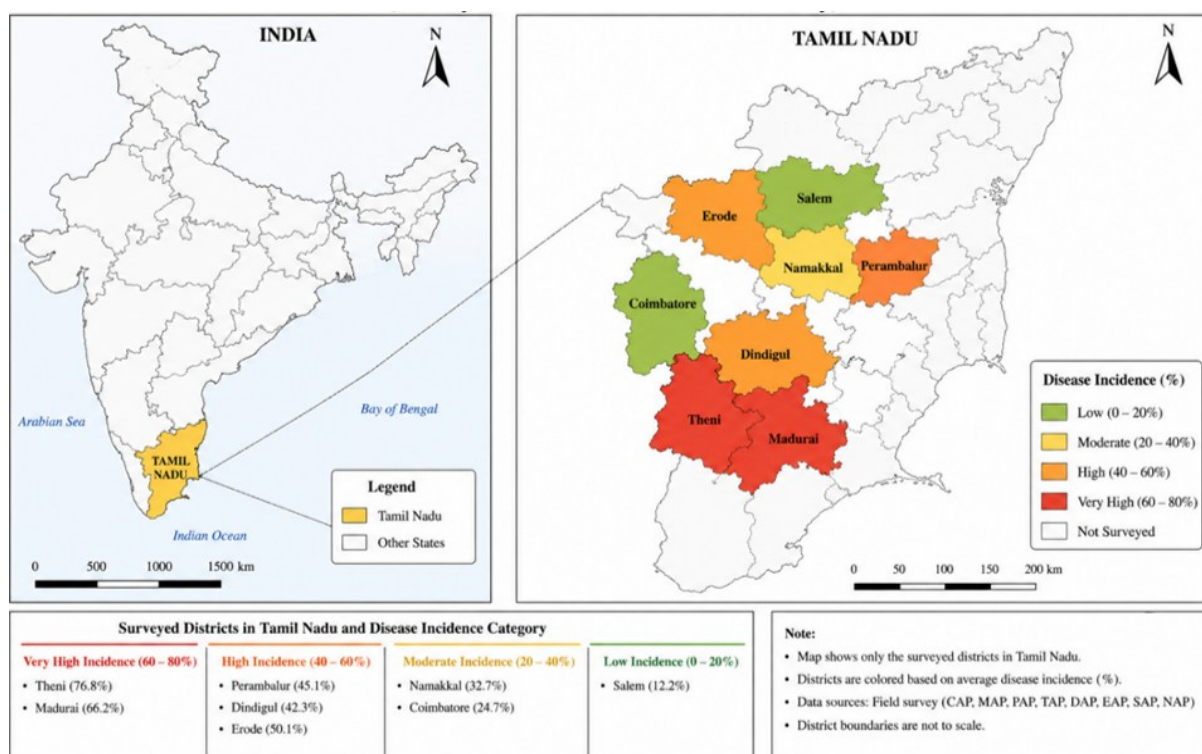


Fig. 2. Spatial distribution of purple blotch disease incidence in Tamil Nadu. [Source: Map adapted from DIVA-GIS]

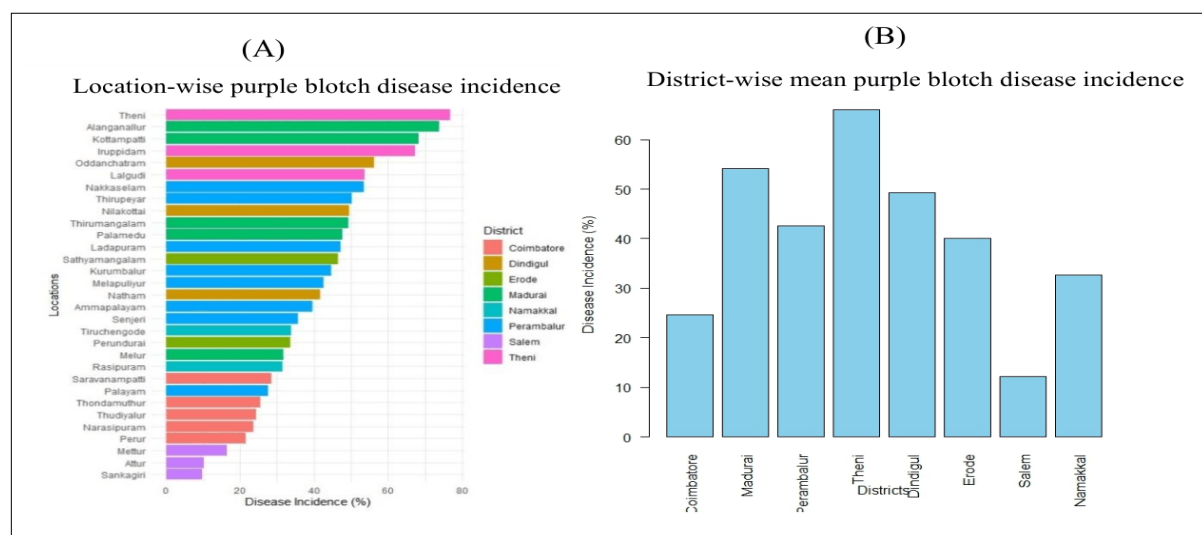


Fig. 3. Purple blotch disease incidence in onion across different locations and districts of Tamil Nadu. (A) Location-wise disease incidence (%) showing variation among surveyed locations; (B) District-wise mean disease incidence (%) indicating overall severity across districts.

Isolation of the pathogen

Leaves showing characteristic symptoms of purple blotch disease were collected from the surveyed areas and used for isolation of the associated pathogen. Following surface sterilisation and incubation on potato dextrose agar (PDA), fungal growth was consistently observed within 5–7 days, indicating the successful recovery of the associated pathogen from infected tissues (Fig. 4). The pathogen readily emerged from the inoculated tissue segments, producing fast-growing colonies. Initially, the colonies appeared greyish to dark olivaceous, which gradually transitioned to dark brown to black pigmentation with age. A total of 31 isolates were obtained and designated according to their geographical origin. A total of 44 symptomatic leaf samples were collected from different onion-growing locations of Tamil Nadu, from which 31 isolates of *A. porri* were successfully recovered, resulting in an isolation frequency of 68.89 %.

Pure cultures were established using single-spore and hyphal-tip isolation techniques to ensure uniformity and eliminate contamination. Distinct differences in colony texture, pigmentation intensity and zonation were observed among isolates, suggesting intraspecific variability. Purified cultures were subsequently maintained on PDA slants at 4 °C for further experimental analyses.

Cultural and morphological identification of the pathogen

The 31 isolates of *A. porri* showed noticeable variation in their cultural and morphological features when grown on PDA medium (Table 2; Fig. 5 & Fig. 6). Colony colour among isolates ranged from greyish white, creamish, light brown and pale white to dark grey, dark olivaceous and black. Isolates such as CAP (NAR)-2, CAP (SAR)-6, MAP (KOT)-8, PAP (NKL)-16 and EAP (SAT)-27 produced dark, dense colonies with prominent concentric zonation, whereas isolates such as CAP (PER)-5, PAP (SEN)-15, SAP (MET)-28 and NAP (TRI)-31 produced lighter, cottony or fluffy colonies with uniform growth patterns. Colony texture varied widely among isolates, including cottony, velvety, fluffy, powdery and dense forms, whereas growth patterns included radial, concentric, circular, uniform and irregular types, indicating substantial phenotypic diversity within the pathogen population.

Microscopic observations revealed that all isolates produced conidia typical of *A. porri*. The conidia were predominantly obclavate, ellipsoidal, or cylindrical in shape and brown in colour. They were produced singly or in short chains and exhibited both transverse and longitudinal septa, giving them a characteristic muriform appearance. Obclavate conidia were commonly observed in isolates such as CAP (NAR)-2, MAP (KOT)-8, PAP (NKL)-16, TAP (LGD)-22 and EAP (SAT)-27, whereas ellipsoidal forms were observed in CAP (THU)-3, PAP (MEL)-13, DAP (NAT)-25 and NAP (RKP)-32 and cylindrical conidia were observed in MAP (ALA)-7 and TAP (THR)-20.

A distinct apical beak, a key diagnostic feature of *A. porri*, was observed in all isolates, although considerable variation in beak length was recorded. Based on morphological observations, isolates were broadly categorised into long, short and moderate (including intermediate forms such as medium to short and moderate to long) beak types. Long beaks were observed in isolates CAP (THU)-3, CAP (THD)-4, CAP (PER)-5, MAP (KOT)-8, MAP (MEL)-9, MAP (TWR)-10, PAP (SEN)-15 and TAP (THR)-20. In contrast, short beaks were recorded in isolates MAP (ALA)-7, MAP (PAL)-11, PAP (LAD)-12, PAP (THR)-17, PAP (PAL)-19, SAP (SAN)-29

and NAP (TRI)-31. The majority of isolates showed moderate to intermediate beak development. These included CAP (NAR)-2, CAP (SAR)-6, PAP (MEL)-13, PAP (AMM)-14, PAP (NKL)-16, PAP (KUR)-18, TAP (UPP)-21, TAP (LGD)-22, DAP (ODC)-23, DAP (NLT)-24, DAP (NAT)-25, EAP (PLR)-26, EAP (SAT)-27, SAP (MET)-28, SAP (ATR)-30 and NAP (RKP)-32.

Quantitative analysis revealed significant variability in conidial dimensions among isolates. Conidial length ranged from 34.5 µm in SAP (SAN)-29 to 80.5 µm in TAP (THR)-20, while breadth ranged from 8.8–14.8 µm. Larger conidia were observed in isolates such as PAP (NKL)-16, MAP (KOT)-8 and TAP (THR)-20, whereas smaller conidia were recorded in SAP (SAN)-29, SAP (ATR)-30 and SAP (MET)-28. The isolates exhibited 3–9 transverse septa and 1–3 longitudinal septa in their conidia. Greater morphological complexity was observed in isolates such as MAP (KOT)-8 and PAP (NKL)-16 due to the presence of higher septation compared to isolates possessing fewer septa. Sporulation varied from low to high among the isolates. High sporulation was observed in isolates such as CAP (NAR)-2, MAP (KOT)-8, PAP (NKL)-16 and EAP (SAT)-27, whereas isolates such as CAP (PER)-5, PAP (SEN)-15, SAP (MET)-28 and NAP (TRI)-31 exhibited comparatively lower sporulation. Moderate sporulation was observed in several intermediate isolates. The variations observed in the cultural and morphological characteristics among the 31 isolates suggest the existence of considerable intraspecific diversity within the *A. porri* population. Based on combined cultural and morphological characteristics, the isolates were grouped into four distinct morphological groups (MG-I to MG-IV), reflecting differences in colony pigmentation, sporulation, conidial dimensions and beak morphology. The observed variability highlights substantial phenotypic diversity within the *A. porri* population of Tamil Nadu.

Molecular characterisation and phylogenetic diversity of *Alternaria porri* isolates

Molecular characterisation of the 31 fungal isolates was carried out through amplification and sequencing of the ITS region. The PCR amplification of the ITS region yielded a clear and consistent amplicon of approximately 560 bp for all isolates, confirming the successful amplification of the target region (Fig. 7). The PCR-amplified products were sequenced and the obtained ITS sequences were analysed using the Basic Local Alignment Search Tool (BLAST) tool available in the NCBI database to identify their closest sequence matches. The BLAST results revealed that all isolates exhibited 98–100 % sequence similarity with *A. porri* reference sequences, thereby confirming the identity of the pathogen associated with purple blotch disease in onion. The high sequence similarity observed among the isolates reflects the conserved nature of the ITS region and further validates the morphological identification of the pathogen. To further validate the species-level identity, PCR amplification was carried out using *A. porri* specific primers. All isolates produced a distinct and uniform amplicon of approximately 118 bp, which is characteristic of *A. porri* (Fig. 8). The absence of non-specific amplification and the consistency of banding patterns across all isolates further confirmed the specificity and reliability of the primers used. The concordance between ITS sequencing and species-specific PCR amplification provides a robust confirmation of pathogen identity. The ITS sequences obtained from all isolates were subsequently submitted to the NCBI GenBank database and accession numbers were assigned to

Table 2. Cultural and morphological characteristics of *Alternaria porri* isolates

Isolate code	Colony colour	Texture	Growth pattern	Conidial shape	Beak	Transverse septa	Longitudinal septa	Sporulation	Length (µm)	Breadth (µm)
CAP (NAR)-2	Greyish black	Velvety	Concentric rings	Obclavate	Moderate	5–8	1–2	High	52.4 (7.2) ^{fg}	11.8 (3.4) ^{ab}
CAP (THU)-3	Greyish white	Cottony	Radial	Ellipsoidal	Long	4–6	1–2	Moderate	48.6 (6.9) ^h	11.2 (3.3) ^{bc}
CAP (THD)-4	Light grey	Fluffy	Circular	Obclavate	Long	5–7	1–2	Moderate	55.8 (7.5) ^{ef}	12.4 (3.5) ^a
CAP (PER)-5	White to grey	Cottony	Uniform	Ellipsoidal	Long	4–6	1	Low	46.2 (6.7) ^h	10.8 (3.2) ^{cd}
CAP (SAR)-6	Grey	Velvety	Concentric	Obclavate	Moderate	5–8	1–2	High	58.9 (7.6) ^{de}	12.1 (3.4) ^{ab}
MAP (ALA)-7	Pale white	Fluffy	Irregular	Cylindrical	Short	3–5	1	Low	72.5 (8.5) ^{ab}	13.6 (3.7) ^a
MAP (KOT)-8	Dark grey	Dense	Radial	Obclavate	Long	6–9	2–3	High	75.8 (8.7) ^a	14.2 (3.8) ^a
MAP (MEL)-9	Brownish black	Powdery	Irregular	Ellipsoidal	Long	5–7	1–2	High	44.5 (6.6) ^h	10.1 (3.1) ^{de}
MAP (TWR)-10	Grey	Velvety	Concentric	Obclavate	Long	6–8	2	High	60.2 (7.8) ^{cd}	12.8 (3.6) ^a
MAP (PAL)-11	White	Cottony	Circular	Ellipsoidal	Short	4–6	1	Low	57.4 (7.6) ^{de}	11.5 (3.3) ^{bc}
PAP (LAD)-12	Greyish white	Fluffy	Uniform	Cylindrical	Short	3–5	1	Low	43.8 (6.5) ^h	9.8 (3.0) ^{ef}
PAP (MEL)-13	Creamish	Cottony	Radial	Ellipsoidal	Moderate	4–6	1	Moderate	50.6 (7.1) ^g	10.9 (3.2) ^{cd}
PAP (AMM)-14	Light brown	Velvety	Concentric	Obclavate	Medium to short	6–8	2	High	63.7 (8.0) ^{bc}	12.9 (3.6) ^a
PAP (SEN)-15	White	Fluffy	Uniform	Ellipsoidal	Long	4–5	1	Low	47.3 (6.8) ^h	10.2 (3.1) ^{de}
PAP (NKL)-16	Dark olivaceous	Dense	Concentric	Obclavate	Medium to short	6–9	2–3	High	78.2 (8.9) ^a	14.5 (3.9) ^a
PAP (THR)-17	Greyish	Cottony	Circular	Ellipsoidal	Short	5–7	1–2	Moderate	61.5 (7.9) ^{cd}	12.6 (3.5) ^a

PAP (KUR)-18	Brownish	Velvety	Irregular	Obclavate	Moderate	6-8	2	High	64.8 (8.1) ^{bc}	13.1 (3.6) ^a
PAP (PAL)-19	Grey	Fluffy	Radial	Ellipsoidal	Short	4-6	1	Moderate	49.2 (7.0) ^g	10.6 (3.2) ^{cd}
TAP (THR)-20	Light grey	Cottony	Uniform	Cylindrical	Long	3-5	1	Low	80.5 (9.0) ^a	14.8 (4.0) ^a
TAP (UPP)-21	Creamy white	Fluffy	Circular	Ellipsoidal	Medium to short	4-6	1	Moderate	70.6 (8.4) ^{ab}	13.5 (3.7) ^a
TAP (LGD)-22	Greyish brown	Velvety	Concentric	Obclavate	Medium to short	6-8	2	High	65.2 (8.1) ^{bc}	12.9 (3.6) ^a
DAP (ODC)-23	Pale white	Cottony	Uniform	Ellipsoidal	Medium to short	3-5	1	Low	66.8 (8.2) ^{bc}	13.2 (3.6) ^a
DAP (NLT)-24	Grey	Powdery	Irregular	Obclavate	Moderate	5-7	1-2	Moderate	60.1 (7.8) ^{cd}	12.4 (3.5) ^{ab}
DAP (NAT)-25	White	Cottony	Circular	Ellipsoidal	Medium to short	4-6	1	Low	52.6 (7.2) ^{fg}	11.3 (3.3) ^{bc}
EAP (PLR)-26	Greyish white	Cottony	Concentric	Obclavate	Moderate to long	5-8	1-2	Moderate	45.9 (6.7) ^h	10.5 (3.2) ^{cd}
EAP (SAT)-27	Dark grey to black	Velvety	Irregular / zonate	Obclavate	Medium to short	6-9	2-3	High	59.8 (7.7) ^{cd}	12.7 (3.5) ^a
SAP (MET)-28	Light grey	Fluffy	Uniform	Ellipsoidal	Medium to short	4-6	1	Low	38.6 (6.2) ⁱ	9.2 (2.9) ^f
SAP (SAN)-29	Dark olivaceous	Velvety	Concentric rings	Obclavate	Short	6-8	2	High	34.5 (5.8) ^j	8.8 (2.8) ^f
SAP (ATR)-30	Brownish grey	Powdery	Irregular	Obclavate	Moderate	5-7	1-2	Moderate	36.2 (6.0) ^j	9.0 (2.9) ^f
NAP (TRI)-31	Creamish white	Cottony	Circular	Ellipsoidal	Short	4-5	1	Low	47.1 (6.8) ^h	10.4 (3.2) ^{cd}
NAP (RKP)-32	Light brown	Fluffy	Radial	Ellipsoidal	Medium to short	4-6	1	Moderate	44.8 (6.6) ^h	10.1 (3.1) ^{de}

* Mean of ten conidia
Values in the parentheses are transformed values.

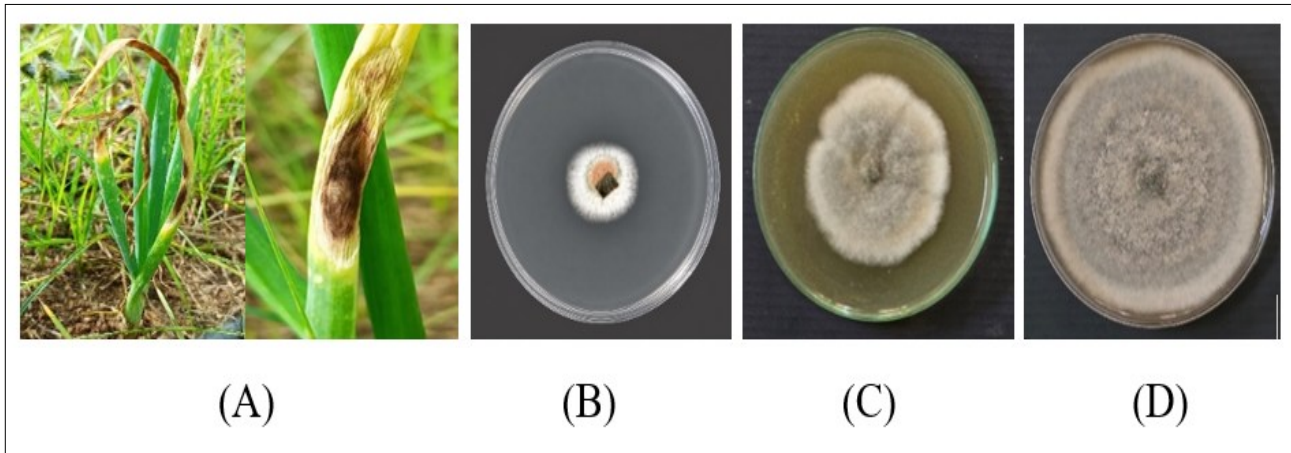


Fig. 4. Isolation of *Alternaria porri* isolates from infected onion leaves: (A) Purple blotch symptoms on onion leaf showing characteristic necrotic lesions; (B) Isolation of the pathogen on potato dextrose agar (PDA) using infected tissue bits; (C) Initial colony development of the fungus on PDA; (D) Fully developed colony exhibiting typical morphology of *Alternaria porri*.



Fig. 5. Morphological variation among *Alternaria porri* isolates collected from different onion-growing regions of Tamil Nadu. (1) CAP (NAR)-2, (2) CAP (THU)-3, (3) CAP (THD)-4, (4) CAP (PER)-5, (5) CAP (SAR)-6, (6) MAP (ALA)-7, (7) MAP (KOT)-8, (8) MAP (MEL)-9, (9) MAP (TWR)-10, (10) MAP (PAL)-11, (11) PAP (LAD)-12, (12) PAP (MEL)-13, (13) PAP (AMM)-14, (14) PAP (SEN)-15, (15) PAP (NKL)-16, (16) PAP (THR)-17, (17) PAP (KUR)-18, (18) PAP (PAL)-19, (19) TAP (THR)-20, (20) TAP (UPP)-21, (21) TAP (LGD)-22, (22) DAP (ODC)-23, (23) DAP (NLT)-24, (24) DAP (NAT)-25, (25) EAP (PLR)-26, (26) EAP (SAT)-27, (27) SAP (MET)-28, (28) SAP (SAN)-29, (29) SAP (ATR)-30, (30) NAP (TRI)-31 and (31) NAP (RKP)-32.

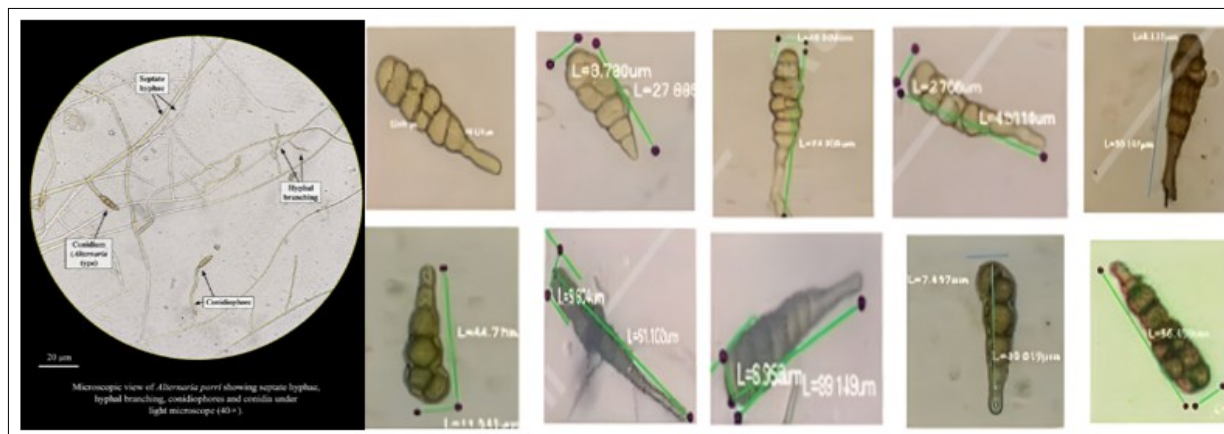


Fig. 6. Morphological variability and micrometric measurements of *Alternaria porri* conidia isolated from onion purple blotch lesions.

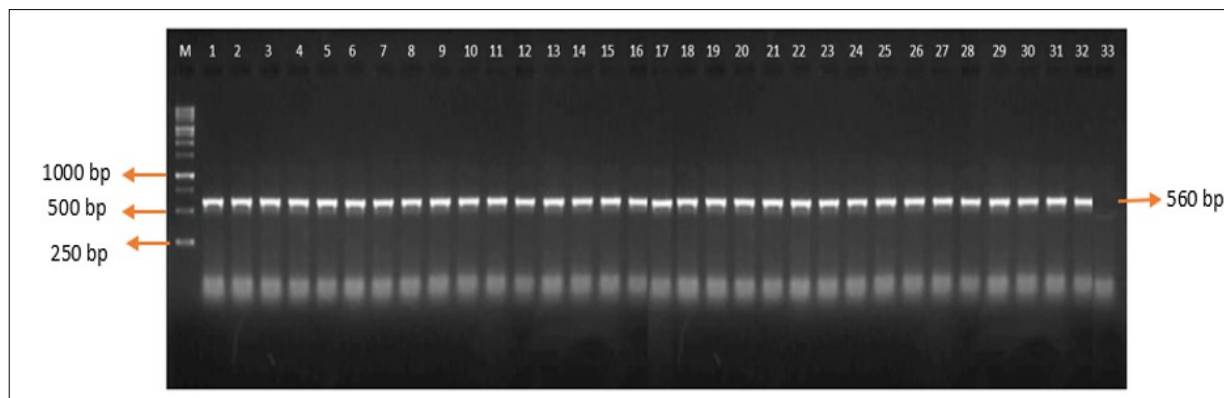


Fig. 7. PCR amplification of internal transcribed spacer (ITS) region of *Alternaria porri* isolates using universal ITS primers (ITS1 and ITS4) whereas as, Lane M: 100 bp DNA ladder; Lane 1: CAP (NAR)-2; Lane 2: CAP (THU)-3; Lane 3: CAP (THD)-4; Lane 4: CAP (PER)-5; Lane 5: CAP (SAR)-6; Lane 6: MAP (ALA)-7; Lane 7: MAP (KOT)-8; Lane 8: MAP (MEL)-9; Lane 9: MAP (TWR)-10; Lane 10: MAP (PAL)-11; Lane 11: PAP (LAD)-12; Lane 12: PAP (MEL)-13; Lane 13: PAP (AMM)-14; Lane 14: PAP (SEN)-15; Lane 15: PAP (NKL)-16; Lane 16: PAP (THR)-17; Lane 17: PAP (KUR)-18; Lane 18: PAP (PAL)-19; Lane 19: TAP (THR)-20; Lane 20: TAP (UPP)-21; Lane 21: TAP (LGD)-22; Lane 22: DAP (ODC)-23; Lane 23: DAP (NLT)-24; Lane 24: DAP (NAT)-25; Lane 25: EAP (PLR)-26; Lane 26: EAP (SAT)-27; Lane 27: SAP (MET)-28; Lane 28: SAP (SAN)-29; Lane 29: SAP (ATR)-30; Lane 30: NAP (TRI)-31; Lane 31: NAP (RKP)-32; Lane 32: +ve Control; Lane 33: Negative control.

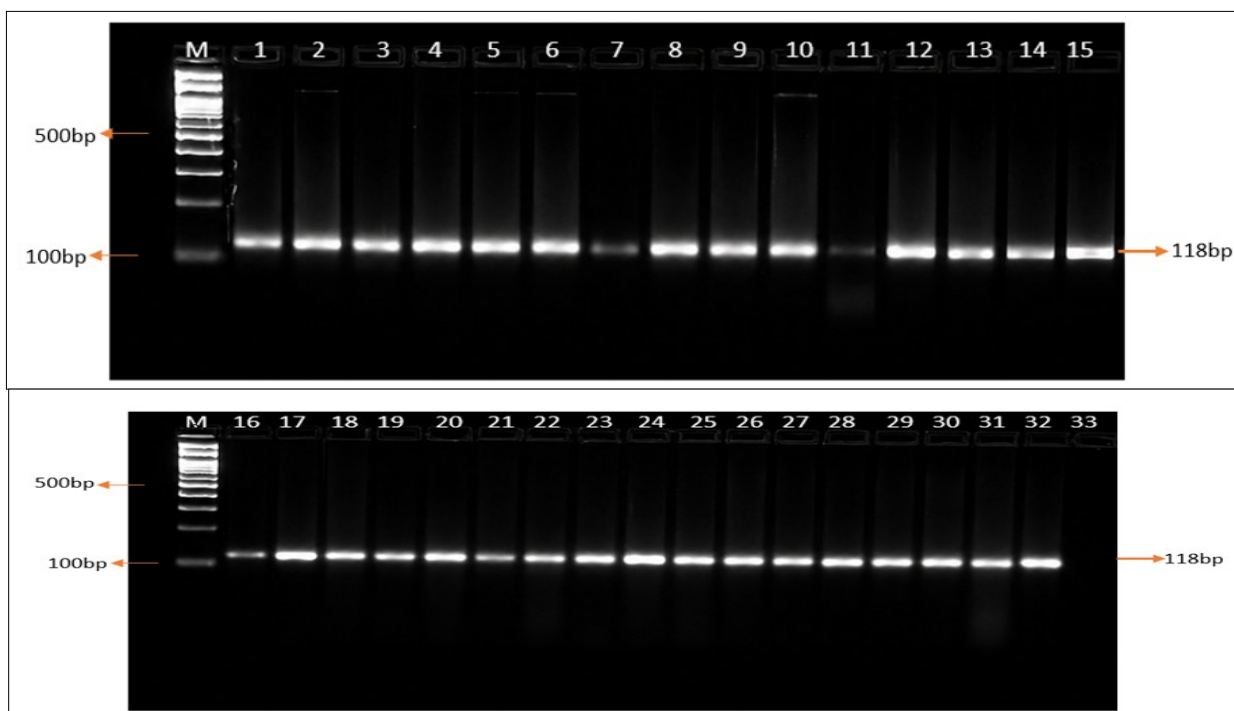


Fig. 8. Confirmation of *Alternaria porri* isolates through species-specific PCR using AsolDelta1/AsolIAlta1 primers. Whereas, Lane M: 100 bp DNA ladder; Lane 1: CAP (NAR)-2; Lane 2: CAP (THU)-3; Lane 3: CAP (THD)-4; Lane 4: CAP (PER)-5; Lane 5: CAP (SAR)-6; Lane 6: MAP (ALA)-7; Lane 7: MAP (KOT)-8; Lane 8: MAP (MEL)-9; Lane 9: MAP (TWR)-10; Lane 10: MAP (PAL)-11; Lane 11: PAP (LAD)-12; Lane 12: PAP (MEL)-13; Lane 13: PAP (AMM)-14; Lane 14: PAP (SEN)-15; Lane 15: PAP (NKL)-16; Lane 16: PAP (THR)-17; Lane 17: PAP (KUR)-18; Lane 18: PAP (PAL)-19; Lane 19: TAP (THR)-20; Lane 20: TAP (UPP)-21; Lane 21: TAP (LGD)-22; Lane 22: DAP (ODC)-23; Lane 23: DAP (NLT)-24; Lane 24: DAP (NAT)-25; Lane 25: EAP (PLR)-26; Lane 26: EAP (SAT)-27; Lane 27: SAP (MET)-28; Lane 28: SAP (SAN)-29; Lane 29: SAP (ATR)-30; Lane 30: NAP (TRI)-31; Lane 31: NAP (RKP)-32; Lane 32: +ve Control; Lane 33: Negative control.

each sequence. A total of 31 isolates were successfully deposited, with accession numbers ranging from PX249769 to PX249807 and PX257939 to PX257949 (Table 3). These accession numbers were derived from the ITS sequence data, ensuring the traceability, reproducibility and public availability of the molecular data.

Phylogenetic analysis based on ITS sequences revealed genetic variability among the *A. porri* isolates collected from different onion-growing regions of Tamil Nadu (Fig. 9). The phylogenetic tree grouped the isolates into four major clades (Clade I–IV) along with reference *A. porri* sequences retrieved from the NCBI GenBank database. Most of the isolates clustered closely with the reference sequences, confirming their identity as *A. porri*. Clade I comprised isolates CAP(NAR)-2, NAP(RKP)-32, PAP(NKL)-16, CAP(THU)-3, EAP(PLR)-26, PAP(LAD)-12, TAP(UPP)-21 and PAP(THR)-17. Clade II included isolates CAP(PER)-5, PAP(AMM)-14, CAP(SAR)-6, DAP(NAT)-25, MAP(PAL)-11, DAP(NLT)-24, PAP(MEL)-13 and SAP(MET)-28. Clade III contained isolates SAP(SAN)-29, CAP(THD)-4, MAP(MEL)-9, TAP(LGD)-22, EAP(SAT)-27, PAP(SEN)-15, PAP(KUR)-18 and DAP(ODC)-23, whereas Clade IV consisted of isolates MAP(ALA)-7, TAP(THR)-20, MAP(KOT)-8, MAP(TWR)-10, PAP(PAL)-19 and NAP(TRI)-31.

The phylogenetic clustering pattern also showed variation in virulence among the isolates. Highly virulent isolates such as MAP(ALA)-7, MAP(KOT)-8 and TAP(THR)-20 were mainly grouped within Clade IV, while moderately and less virulent isolates were distributed across Clades I, II and III. The reference *A. porri* sequences grouped within the same clade, further validating the molecular identification of the isolates. In contrast, the outgroup species *Stemphylium vesicarium* and *Cladosporium* spp. formed distinct separate branches away from all *A. porri* isolates.

Table 3. GenBank accession numbers of *Alternaria porri* isolates

Sl. No.	Isolate code	Accession number
1	CAP (NAR)-2	PX249769
2	CAP (THU)-3	PX249770
3	CAP (THD)-4	PX249771
4	CAP (PER)-5	PX249772
5	CAP (SAR)-6	PX249773
6	MAP (ALA)-7	PX249774
7	MAP (KOT)-8	PX249775
8	MAP (MEL)-9	PX249776
9	MAP (TWR)-10	PX249777
10	MAP (PAL)-11	PX249778
11	PAP (LAD)-12	PX249798
12	PAP (MEL)-13	PX249799
13	PAP (AMM)-14	PX249800
14	PAP (SEN)-15	PX249801
15	PAP (NKL)-16	PX249802
16	PAP (THR)-17	PX249803
17	PAP (KUR)-18	PX249804
18	PAP (PAL)-19	PX249805
19	TAP (THR)-20	PX249806
20	TAP (UPP)-21	PX249807
21	TAP (LGD)-22	PX257939
22	DAP (ODC)-23	PX257940
23	DAP (NLT)-24	PX257941
24	DAP (NAT)-25	PX257942
25	EAP (PLR)-26	PX257943
26	EAP (SAT)-27	PX257944
27	SAP (MET)-28	PX257945
28	SAP (SAN)-29	PX257946
29	SAP (ATR)-30	PX257947
30	NAP (TRI)-31	PX257948
31	NAP (RKP)-32	PX257949

The bootstrap support values observed at branch nodes indicated the reliability of clustering among the isolates. Overall, the phylogenetic analysis revealed moderate intraspecific genetic diversity among the *A. porri* isolates associated with purple blotch disease of onion, reflecting the genetic variability present within pathogen populations across different onion-growing regions of Tamil Nadu.

Pathogenic variability of *Alternaria porri* isolates

All the *A. porri* isolates tested under pot culture conditions were pathogenic and produced characteristic purple blotch symptoms on small onion CO-5 leaves, thereby confirming their pathogenic nature. Initial symptoms appeared as small water-soaked lesions which gradually enlarged into elliptical purple necrotic lesions surrounded by chlorotic halos, eventually leading to blighting and drying of leaves. Symptom development was observed within 5–7 days after inoculation, whereas no symptoms were observed in uninoculated control plants (Fig. 10).

Significant variability in pathogenicity was observed among the isolates based on PDI values (Table 4). Based on PDI values, the isolates were categorised as highly virulent (> 60 % PDI), virulent (50–60 % PDI), moderately virulent (30–50 % PDI) and mildly virulent (< 30 % PDI) to facilitate the assessment of pathogenic variability among the isolates. Among the isolates, MAP (ALA)-7 recorded the maximum disease severity of 76.80 % followed by TAP (THR)-20 (74.20 %) and MAP (KOT)-8 (71.20 %), indicating their highly virulent nature. These isolates produced severe necrotic lesions, extensive chlorosis and rapid leaf drying under pot culture conditions. TAP (UPP)-21 also exhibited high virulence with a PDI of 69.40 %.

Several isolates showed virulent reactions with moderate to high disease severity. DAP (ODC)-23 recorded 58.70 % disease severity followed by TAP (LGD)-22 (55.90 %), PAP (NKL)-16 (55.70 %), PAP (THR)-17 (52.60 %), MAP (TWR)-10 (52.40 %), DAP (NLT)-24 (51.80 %), MAP (PAL)-11 (50.10 %) and PAP (LAD)-12 (49.50 %). These isolates produced comparatively larger lesions with moderate blighting symptoms.

Moderately virulent isolates included EAP (SAT)-27 (48.20 %), PAP (KUR)-18 (46.90 %), PAP (MEL)-13 (44.80 %), DAP (NAT)-25 (43.60 %), PAP (AMM)-14 (41.30 %), PAP (SEN)-15 (37.80 %), NAP (TRI)-31 (35.60 %), EAP (PLR)-26 (35.20 %), MAP (MEL)-9 (33.50 %) and NAP (RKP)-32 (33.10 %). These isolates produced moderate lesion development with restricted chlorosis and comparatively lesser leaf damage.

Mildly virulent isolates such as CAP (SAR)-6, PAP (PAL)-19, CAP (THD)-4, CAP (THU)-3, CAP (NAR)-2 and CAP (PER)-5 recorded disease severity ranging from 22.80–30.20 %. These isolates induced smaller lesions with limited symptom progression. The least virulent isolates were SAP (MET)-28, SAP (ATR)-30 and SAP (SAN)-29, which recorded minimum disease severity of 18.40, 12.10 and 11.20 % respectively and produced very small restricted lesions with minimal chlorosis.

Re-isolation of the pathogen from artificially infected leaves yielded fungal colonies morphologically similar to the original isolates, thereby fulfilling Koch's postulates and confirming *A. porri* as the causal organism of purple blotch disease in onion. The results clearly demonstrated considerable pathogenic variability among the *A. porri* isolates collected from different onion-growing regions of Tamil Nadu.

Table 4. Comparative summary of morphological diversity, pathogenicity and phylogenetic grouping of *Alternaria porri* isolates collected from major onion-growing regions of Tamil Nadu

Isolate code	Location	Field PDI (%)	In vivo PDI (%)	Morphological group	ITS clade
CAP (NAR)-2	Narasipuram	23.6 (29.1) ^d	25.4 ^{**} (30.3) ^d	MG-IV	Clade I
CAP (THU)-3	Thudiyalur	24.3 (29.5) ^d	26.1 ^{**} (30.7) ^d	MG-I	Clade I
CAP (THD)-4	Thondamuthur	25.5 (30.3) ^d	27.3 ^{**} (31.5) ^d	MG-II	Clade III
CAP (PER)-5	Perur	21.5 (27.6) ^d	22.8 ^{**} (28.5) ^d	MG-I	Clade II
CAP (SAR)-6	Saravanampatti	28.6 (32.3) ^d	30.2 ^{**} (33.3) ^d	MG-III	Clade II
MAP (ALA)-7	Alanganallur	73.8 (59.2) ^a	76.8 ^{*****} (61.2) ^a	MG-IV	Clade IV
MAP (KOT)-8	Kottampatti	68.3 (55.7) ^a	71.2 ^{*****} (57.6) ^a	MG-IV	Clade IV
MAP (MEL)-9	Melur	31.8 (34.3) ^c	33.5 ^{***} (35.4) ^c	MG-III	Clade III
MAP (TWR)-10	Thirumangalam	49.2 (44.6) ^b	52.4 ^{****} (46.4) ^b	MG-II	Clade IV
MAP (PAL)-11	Palamedu	47.7 (43.6) ^b	50.1 ^{****} (45.1) ^b	MG-I	Clade II
PAP (LAD)-12	Ladapuram	47.3 (43.4) ^b	49.5 ^{****} (44.7) ^b	MG-I	Clade I
PAP (MEL)-13	Melapuliyur	42.6 (40.7) ^c	44.8 ^{***} (42.0) ^c	MG-I	Clade II
PAP (AMM)-14	Ammapalayam	39.5 (39.0) ^c	41.3 ^{***} (39.9) ^c	MG-III	Clade II
PAP (SEN)-15	Senjeri	35.6 (36.7) ^c	37.8 ^{***} (37.9) ^c	MG-I	Clade III
PAP (NKL)-16	Nakkasalam	53.4 (47.0) ^b	55.7 ^{****} (48.3) ^b	MG-IV	Clade I
PAP (THR)-17	Thirupeyar	50.2 (45.1) ^b	52.6 ^{****} (46.5) ^b	MG-II	Clade I
PAP (KUR)-18	Kurumbalur	44.6 (41.9) ^c	46.9 ^{***} (43.2) ^c	MG-III	Clade III
PAP (PAL)-19	Palayam	27.5 (31.6) ^d	29.4 ^{**} (32.8) ^d	MG-II	Clade III
TAP (THR)-20	Theni	76.8 (61.2) ^a	74.2 ^{*****} (59.5) ^a	MG-II	Clade IV
TAP (UPP)-21	Iruppidam	67.3 (55.1) ^a	69.4 ^{*****} (56.4) ^a	MG-I	Clade I
TAP (LGD)-22	Lalgudi	53.8 (47.3) ^b	55.9 ^{****} (48.4) ^b	MG-III	Clade III
DAP (ODC)-23	Oddanchatram	56.2 (48.6) ^b	58.7 ^{****} (50.0) ^b	MG-II	Clade III
DAP (NLT)-24	Nilakottai	49.6 (44.8) ^b	51.8 ^{****} (46.0) ^b	MG-II	Clade II
DAP (NAT)-25	Natham	41.7 (40.2) ^c	43.6 ^{***} (41.3) ^c	MG-I	Clade II
EAP (PLR)-26	Perundurai	33.6 (35.5) ^c	35.2 ^{***} (36.4) ^c	MG-II	Clade I
EAP (SAT)-27	Sathyamangalam	46.5 (42.9) ^b	48.2 ^{****} (44.0) ^b	MG-IV	Clade III
SAP (MET)-28	Mettur	16.5 (23.9) ^e	18.4 [*] (25.4) ^e	MG-II	Clade II
SAP (SAN)-29	Sankagiri	9.8 (18.3) ^e	11.2 [*] (19.5) ^e	MG-IV	Clade II
SAP (ATR)-30	Attur	10.3 (18.7) ^e	12.1 [*] (20.4) ^e	MG-III	Clade III
NAP (TRI)-31	Tiruchengode	33.8 (35.6) ^c	35.6 ^{***} (36.7) ^c	MG-I	Clade IV
NAP (RKP)-32	Rasipuram	31.6 (34.2) ^c	33.1 ^{***} (35.1) ^c	MG-III	Clade I
CD ($p = 0.05$)		1.76	1.75		
SEd		0.88	0.87		

Values in parentheses are arc-sine transformed values.

Means followed by different letters differ significantly at $p \leq 0.05$.

MG-I: White/creamy colonies; MG-II: Grey/light grey colonies; MG-III: Brownish colonies; MG-IV: Dark grey/olivaceous colonies; *****: Highly virulent; ****: Virulent; ***: Moderately virulent; **: Mildly virulent; *: Least virulent.

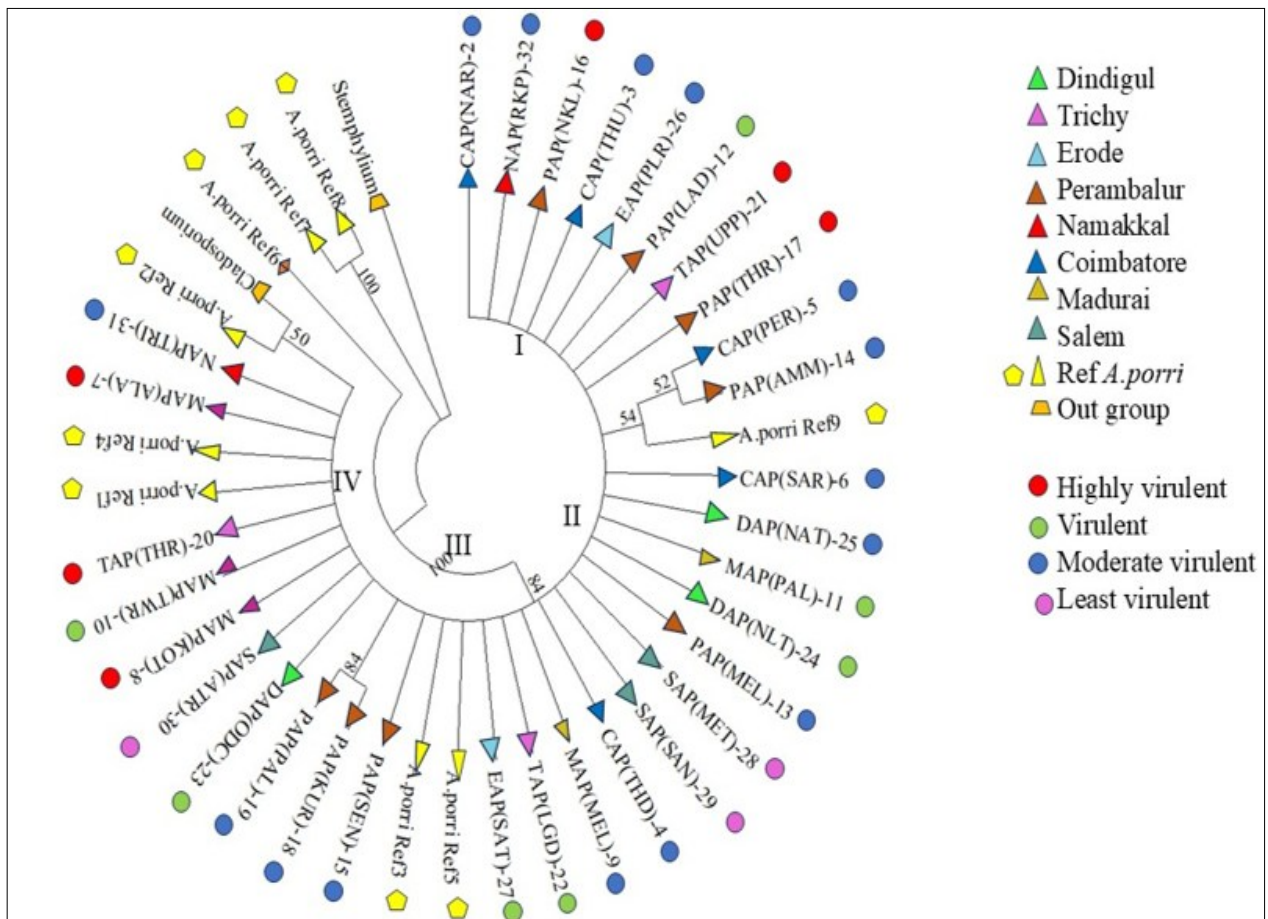


Fig. 9. Phylogenetic relationship among *Alternaria porri* isolates based on internal transcribed spacer (ITS) sequence analysis showing clustering of isolates into four major clades. Colored triangles indicate geographical origin of isolates, whereas colored circles represent virulence reaction of the isolates collected from different onion-growing regions of Tamil Nadu. Bootstrap values are shown at branch nodes.

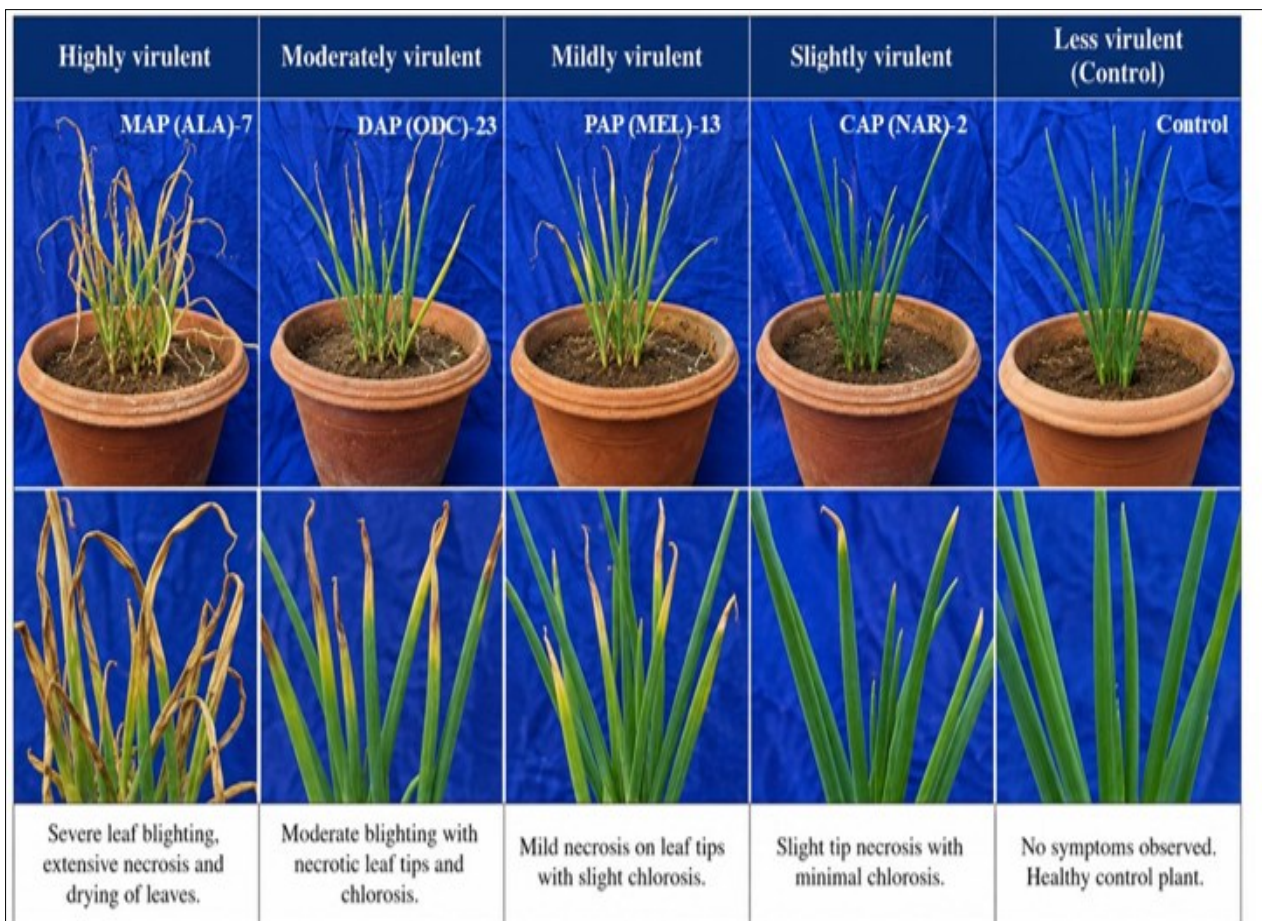


Fig. 10. Pathogenicity assay of *Alternaria porri* isolates on small onion cv. CO-5 under pot culture conditions showing variation in virulence reactions ranging from highly virulent to least virulent isolates.

Discussion

The present investigation revealed considerable variability in purple blotch disease severity across the major onion-growing regions of Tamil Nadu, with PDI values ranging from 9.80–76.80 %. Higher disease incidence recorded in districts such as Theni and Madurai indicates the prevalence of highly favorable environmental conditions for disease establishment and pathogen multiplication in these regions. Environmental factors such as prolonged leaf wetness, high relative humidity and moderate temperature are known to enhance conidial germination, sporulation and infection efficiency in *A. porri*, thereby increasing disease severity under field conditions. In contrast, comparatively lower disease incidence observed in Salem and Coimbatore Districts may be associated with less favourable climatic conditions and differences in local crop management practices. Similar regional variability in purple blotch incidence and disease severity has also been reported previously, emphasising the role of environmental conditions, inoculum pressure and host susceptibility in influencing disease epidemiology (8, 18).

Considerable variation in cultural and morphological characteristics was observed among the 31 isolates of *A. porri*, indicating substantial phenotypic diversity within the pathogen population. Differences in colony pigmentation, growth pattern, texture, sporulation and conidial morphology suggest that the isolates have undergone adaptation to diverse agro-climatic conditions prevailing across different onion-growing regions. The observed variability in conidial size, septation and beak morphology may also contribute to differences in sporulation efficiency, survival ability and infection potential among the isolates. Larger conidia with higher septation observed in certain highly virulent isolates may indicate enhanced reproductive capacity and pathogenic fitness under favourable environmental conditions. Similar morphological variability among *A. porri* isolates has also been previously reported, highlighting significant differences in colony morphology, pigmentation and sporulation among isolates collected from different geographical regions (8, 19–21). Comparable variability in colony morphology, sporulation and pathogenic aggressiveness among *A. porri* isolates has also been reported from onion-growing regions of Maharashtra and Odisha, suggesting geographical adaptation and diversification of pathogen populations across India. Morphological diversification in *Alternaria* species has also been associated with ecological adaptability and pathogenic specialisation (22). Such phenotypic diversity within pathogen populations is epidemiologically important because it may influence survival, dispersal efficiency and disease development under varying environmental conditions.

The present study also demonstrated substantial pathogenic variability among the *A. porri* isolates collected from different onion-growing regions of Tamil Nadu. Significant differences in disease severity under pot culture conditions clearly indicated the existence of virulence diversity within the pathogen population. Highly virulent isolates such as MAP (ALA)-7, MAP (KOT)-8 and TAP (THR)-20 produced severe necrotic lesions, extensive chlorosis and rapid leaf blighting, whereas isolates from Salem region exhibited comparatively lower virulence. Such differences in pathogenic behaviour may be associated with variation in aggressiveness, sporulation efficiency, host colonisation ability and adaptation to local environmental conditions. Environmental factors such as temperature, humidity and leaf wetness duration

may further influence pathogen aggressiveness and epidemic development by enhancing infection efficiency and sporulation potential. Similar observations on pathogenic variability, aggressiveness and lesion development among *A. porri* isolates have also been reported previously (8, 23–25). Environmental conditions were also found to strongly influence the growth, sporulation and pathogenic potential of *A. porri* (26). The clustering of highly virulent isolates predominantly within Clade IV and dark pigmented morphological groups further suggests a possible association between pathogenicity, phenotypic variability and phylogenetic grouping. Such variability within pathogen populations is highly significant from an epidemiological perspective because highly aggressive isolates may contribute to rapid disease spread, severe epidemics and increased yield loss under favourable environmental conditions. Therefore, understanding pathogenic diversity among *A. porri* isolates is essential for identifying durable sources of host resistance and developing effective region-specific disease management strategies.

The integrated analysis of morphology, pathogenicity and phylogenetic diversity revealed a clear relationship among phenotypic variability, virulence behaviour and molecular grouping of the isolates. The categorisation of isolates into four morphological groups and four major ITS clades indicates the existence of moderate intraspecific diversity within the *A. porri* population associated with onion purple blotch disease. The predominance of highly virulent isolates within MG-IV and Clade IV suggests that certain phenotypic and genetic traits may contribute to enhanced aggressiveness and adaptation. Such diversity may arise due to mutation, environmental selection pressure and continuous interaction between the pathogen and host across different agro-ecological regions. Similar observations on morphological and pathogenic diversity among *Alternaria* isolates have also been reported previously, indicating that phenotypic variability often reflects underlying genetic diversification within pathogen populations (8, 22, 25). The existence of such diversity within *A. porri* populations highlights the dynamic evolutionary nature of the pathogen and emphasises the necessity for continuous surveillance and variability studies for sustainable disease management programmes.

Molecular characterisation using ITS region amplification and sequencing provided reliable confirmation of pathogen identity. All isolates produced a consistent ITS amplicon of approximately 560 bp and exhibited 98–100 % sequence similarity with reference *A. porri* sequences available in the NCBI database. The successful amplification obtained using species-specific primers further confirmed the identity of the pathogen isolates. The use of ITS markers and Alt a1 gene-specific primers provides a reliable and rapid approach for accurate identification of *Alternaria* species associated with onion diseases. Similar successful utilisation of ITS-based molecular identification in *Alternaria* species has also been reported previously (8, 16, 17, 25). Accurate molecular identification is particularly important for differentiating closely related *Alternaria* species and for understanding pathogen diversity associated with disease outbreaks. Phylogenetic analysis based on ITS sequences revealed moderate genetic diversity among the isolates and grouped them into four major clades. The clustering pattern observed among the isolates suggests ongoing diversification and adaptation of *A. porri* populations under different ecological conditions. The association of highly virulent isolates with specific

phylogenetic clades may indicate the possible emergence of genetically adapted populations with enhanced aggressiveness. Similar phylogenetic variability among onion-associated *Alternaria* isolates has also been reported previously, demonstrating detectable genetic divergence and pathogenic specialisation among *Alternaria* populations (22, 26). The moderate genetic variability observed in the present study may influence host-pathogen interaction, aggressiveness and disease epidemiology under field conditions. Therefore, continuous monitoring of pathogen diversity and population structure is essential for developing effective breeding programmes and long-term disease management strategies against purple blotch disease of onion.

Conclusion

The present investigation demonstrated significant variability among *Alternaria porri* isolates associated with purple blotch disease of onion in Tamil Nadu. Molecular analysis confirmed the identity of the pathogen and revealed moderate genetic diversity among the isolates. The findings provide a useful foundation for disease surveillance, resistance screening and the development of sustainable management strategies.

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Authors' contributions

SM conceived the study, drafted the manuscript and coordinated the overall preparation of the work. KK critically reviewed, edited and supervised the manuscript preparation, providing important scientific inputs throughout the study. MK, HS, BA, AR, RN, MP and PS contributed to manuscript writing, review and corrections, while RS and MBN assisted with statistical analysis, manuscript alignment and formatting. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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During the preparation of the manuscript, the authors used Grammarly for language editing and improvement of readability. After using this tool, the authors carefully reviewed and edited the manuscript and take full responsibility for the scientific content of the publication.

References

1. Agricultural and Processed Food Products Export Development Authority (APEDA). Fresh onion export and production statistics [Internet]. https://apeda.gov.in/apedawebsite/SubHead_Products/Onion.htm
2. Ministry of Agriculture and Farmers Welfare, Government of India. Horticulture statistics at a glance 2024–25 [Internet]. New Delhi: Government of India; 2025. <https://agricoop.nic.in>
3. Ministry of Agriculture and Farmers Welfare, Government of India. Horticulture statistics at a glance 2024. New Delhi: Government of India; 2024. <https://agricoop.nic.in>
4. Directorate of Economics and Statistics, Government of India. First advance estimates of production of horticultural crops 2023–24. New Delhi: Ministry of Agriculture; 2024. <https://dacnet.nic.in>
5. Tamil Nadu Agricultural University (TNAU). Crop production guide: onion (small onion/shallot). Coimbatore: TNAU; 2023. <https://agritech.tnau.ac.in>
6. Department of Horticulture and Plantation Crops, Government of Tamil Nadu. Policy note and statistical handbook 2023–24. Chennai: Government of Tamil Nadu; 2024. <https://www.tnhorticulture.tn.gov.in>
7. Asghar M, Atiq M, Ali MU, Kachelo GA, Khan NA, Naveed K, et al. Investigating the biochemical variations in onion leaves due to purple blotch disease and its management through induced resistance. *PLoS One*. 2025;20(5). <https://doi.org/10.1371/journal.pone.0323067>
8. Ravichandra GK, Ekabote SD, Bharath M, Hariprasad GN, Ramesh AN, Adivappar N, et al. Morpho-molecular and pathogenic variability of *Alternaria porri* isolates causing onion purple blotch disease in Southern Karnataka, India. *Arch Phytopathol Plant Prot*. 2025;58(9): 511–535. <https://doi.org/10.1080/03235408.2025.2500115>
9. Kumar D, Godara SL, Sheshma MK. Physiological studies on *Alternaria porri* causing purple blotch of onion under *in vitro* conditions. *J Agric Ecol*. 2023; 17:109–112. <https://doi.org/10.58628/JAE-2317-320>
10. Mayee CD, Datar VV. Phytopathometry. Technical Bulletin-I (Special Bulletin-3). Parbhani: Marathwada Agricultural University; 1986.
11. Wheeler BEJ. An introduction to plant diseases. London: John Wiley and Sons; 1969.
12. Dhingra OD, Sinclair JB. Basic plant pathology methods. Boca Raton: CRC Press; 1985.
13. Simmons EG. *Alternaria*: an identification manual. Utrecht: CBS Fungal Biodiversity Centre; 2007.
14. Yessimseitova A, Abdrakhmanova A, Tokbergenova Z, Abdullaeva B, Muranets A, Nurtaza A, et al. Identification and characterization of *Alternaria* species causing early blight on tomato in Kazakhstan. *Agronomy*. 2025;15(5):1251. <https://doi.org/10.3390/agronomy15051251>
15. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*. 1987;19:11–15.
16. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315–22.
17. Pavón MÁ, González I, Pegels N, Martín R, García T. PCR detection and identification of *Alternaria* species-groups in processed foods based on the genetic marker Alt a 1. *Food Control*. 2010;21:1745–56. <https://doi.org/10.1016/j.foodcont.2010.08.004>
18. Agrios GN. Plant pathology. 5th ed. Burlington: Elsevier Academic Press; 2005.
19. Dar AA, Sharma S, Mahajan R, Mushtaq M, Salathia A, Ahamad S, et al. Overview of purple blotch disease and understanding its

- management through chemical, biological and genetic approaches. *J Integr Agric.* 2020;19(12):3013–3024. [https://doi.org/10.1016/S2095-3119\(20\)63285-3](https://doi.org/10.1016/S2095-3119(20)63285-3)
20. Mohsin SM, Islam MR, Ahmmed ANF, Nisha HAC, Hasanuzzaman M. Cultural, morphological and pathogenic characterization of *Alternaria porri* causing purple blotch of onion. *Not Bot Horti Agrob.* 2016;44(1):222–227. <https://doi.org/10.15835/nbha44110110>
 21. Santhiya S, Hedau NK, Thakur A, Singh A, Islam S, Dev R, et al. Recent advancement and perspectives of molecular markers for resistance against purple blotch disease in onion (*Allium cepa*). *Discov Plants.* 2026;3(1):93. <https://doi.org/10.1007/s44372-026-00565-5>
 22. Wang F, Saito S, Michailides TJ, Xiao CL. Phylogenetic, morphological and pathogenic characterization of *Alternaria* species associated with fruit rot of mandarin in California. *Plant Dis.* 2021;105(9):2606–2617. <https://doi.org/10.1094/PDIS-10-20-2145-RE>
 23. Ahmadpour A, Ghosta Y, Alavi Z, Alavi F, Hamidi LM, Rampelotto PH. Morphological and molecular characterization of a new section and two new species of *Alternaria* from Iran. *Life.* 2025;15(6):870. <https://doi.org/10.3390/life15060870>
 24. Behera S, Das S. Effect of weather variables on the biology of *Alternaria porri* causing purple blotch disease of onion under controlled environmental condition. *J Pharmacogn Phytochem.* 2018;7(4):315–19.
 25. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci USA.* 2012;109(16):6241–6246. <https://doi.org/10.1073/pnas.1117018109>
 26. Sharma R, Mishra R, Joshi RK. A highly contiguous genome sequence of *Alternaria porri* isolate Apn-Nashik causing purple blotch disease in onion. *BMC Genomic Data.* 2024;25(1):95. <https://doi.org/10.1186/s12863-024-01276-0>

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