

# PHENOTYPIC AND MOLECULAR VARIABILITY AMONG *ALTERNARIA SOLANI* CAUSING POTATO EARLY BLIGHT IN GUJARAT, INDIA

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**ABSTRACT:** Early blight, caused by *Alternaria solani*, is an important foliar disease of potato and can cause substantial yield losses. This study assessed phenotypic and molecular variability among thirty-six *A. solani* isolates collected from major potato-growing regions of Gujarat, India. Thirty-six *Alternaria* isolates obtained from six major potato-growing districts of Gujarat exhibited substantial cultural, morphological and molecular variability. Conidial dimensions varied from 12.5–89.4 × 4.6–30.5 µm on potato dextrose agar and from 12.1–82.4 × 4.1–27.7 µm on Richard's agar. ITS1/ITS4 amplification and sequencing of representative isolate As1 produced an amplicon of approximately 540 bp and showed 98–99% similarity with *A. solani* sequences in Gen Bank, supporting its identification. RAPD analysis of all 36 isolates using five informative primers generated 30 scorable loci, of which 28 were polymorphic (93.3%). UPGMA analysis resolved the isolates into two major clusters. AMOVA attributed 20% of molecular variation to differences between the two geographic populations and 80% to variation within populations. The results demonstrate substantial phenotypic and RAPD-based genetic variability in the Gujarat isolate collection and provide baseline information relevant to pathogen surveillance, resistance breeding and early-blight management.

**KEYWORDS:** *Alternaria solani*, early blight, pathogen diversity, ITS-rDNA, RAPD marker

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important food crops and an important source of carbohydrates, vitamins and minerals. In India, potato cultivation contributes substantially to agricultural production and food security. Its productivity, however, is constrained by several diseases, among which early blight caused by *Alternaria solani* is economically important. The pathogen also infects other solanaceous crops, including tomato, brinjal

and chilli. It can survive between cropping seasons in infected plant debris, infected tubers and soil, thereby providing inoculum for subsequent crops (Rotem, 1998). Early blight affects leaves, stems and tubers and consequently reduces both yield and marketable quality.

Yield losses of 30–50% have been reported under severe disease conditions (Rouse, 1985), while Haware (1968) reported losses of up to 40%. Several *Alternaria* species infect solanaceous crops. Large-spored species include

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*A. solani*, *A. grandis*, *A. protenta* and *A. linariae*, while *A. alternata* and the *A. arborescens* complex represent small-spored species. *Alternaria solani* is the predominant cause of potato early blight, although other large-spored species may also cause similar symptoms (Adhikari *et al.*, 2020; Ivanovic *et al.*, 2022).

The disease initially appears as small, dark-brown lesions that enlarge and develop characteristic concentric rings, producing a target-like appearance. Progressive lesion development causes premature defoliation, reduces the photosynthetically active leaf area and ultimately diminishes tuber development and yield (Van der Waals *et al.*, 2001). Identification of *A. solani* has traditionally been based on cultural and morphological characteristics, including colony colour, colony texture, growth pattern, conidial dimensions and septation (Ellis, 1971). However, these phenotypic characteristics may vary with the isolate, culture medium, temperature, light regime and age of the culture. Morphological observations alone may therefore be insufficient to assess relationships among isolates.

The internal transcribed spacer region of ribosomal DNA is widely used for fungal identification and DNA barcoding (Nilsson *et al.*, 2009; Schoch *et al.*, 2012). Nevertheless, the ITS region may have limited discriminatory power among closely related species of *Alternaria*, and multilocus or genome-wide markers generally provide stronger taxonomic and population-level resolution (Adhikari *et al.*, 2021; Schmey *et al.*, 2024). Random amplified polymorphic DNA markers can provide an initial and relatively inexpensive assessment of DNA polymorphism within a pathogen collection, provided that amplification conditions are standardized and only clear and reproducible bands are scored. The combined evaluation of cultural, morphological and molecular

characteristics can therefore provide a broader understanding of variability within an *A. solani* collection than any single approach.

Early blight is widely observed in the principal potato-growing regions of Gujarat. However, comparative information on the cultural, morphological and genetic variability of *A. solani* isolates from geographically separated locations in the state remains limited. This constitutes an important knowledge gap because variability within a pathogen population may influence disease development, host-pathogen interactions, identification of resistant germplasm and the durability of disease-management practices. Characterization of isolates representing different potato-growing regions is therefore required to determine the extent of variability present in the local pathogen population and to provide baseline information for future epidemiological and resistance-breeding studies.

Accordingly, the present investigation aimed to characterize *A. solani* isolates collected from the major potato-growing districts of Gujarat. The specific objectives were: (i) to compare the cultural characteristics of the isolates on potato dextrose agar and Richard's agar (ii) to compare the morphological characteristics of the isolates on potato dextrose agar and Richard's agar (iii) to support the identity of a representative isolate through ITS-rDNA sequencing (iv) to assess RAPD-based genetic diversity and relationships among the 36 isolates.

## MATERIALS AND METHODS

### Collection of Diseased Samples/Isolates

Thirty-six isolates of *A. solani* were collected during November 2022 from major potato-growing districts of Gujarat, namely Anand, Kheda, Gandhinagar, Aravalli, Sabarkantha and Banaskantha (Table 1).

**Table 1. Sources of isolates of *A. solani* collected from the major potato growing regions of Gujarat**

| Sr. No. | Isolate No. | Region         | District    | Taluka    | Place          | GPS Location                |
|---------|-------------|----------------|-------------|-----------|----------------|-----------------------------|
| 1       | As1         | Middle Gujarat | Anand       | Anand     | Anand-1        | 22.536471°N<br>72.980321°W  |
| 2       | As2         |                |             |           | Anand-2        | 22.536636°N<br>72.982272°W  |
| 3       | As3         |                |             |           | Bakrol         | 22.573503°N<br>72.910713°W  |
| 4       | As4         |                |             |           | Lambhvel       | 22.591490°N<br>72.937305°W  |
| 5       | As5         |                |             |           | Boriyavi       | 22.616162°N<br>72.932162°W  |
| 6       | As6         |                |             |           | Khambhodaj     | 22.575454°N<br>73.087227°W  |
| 7       | As7         |                | Kheda       | Nadiad    | Narsanda       | 22.627235°N<br>72.887928°W  |
| 8       | As8         |                |             |           | Chaklasi       | 22.657834°N<br>72.944251°W  |
| 9       | As9         |                |             |           | Kanjari        | 22.622818°N<br>72.913822°W  |
| 10      | As10        |                |             |           | Vadtal         | 22.5980023°N<br>72.872672°W |
| 11      | As11        | North Gujarat  | Gandhinagar | Kathalal  | Shahpur        | 22.902035°N<br>73.017003°W  |
| 12      | As12        |                |             | Kapadvanj | Unda ni muvadi | 22.980844°N<br>73.010760°W  |
| 13      | As13        |                |             |           | Kevadiya       | 23.083597°N<br>72.992592°W  |
| 14      | As14        |                | Gandhinagar | Dehgam    | Khanpur        | 23.233534°N<br>72.896837°W  |
| 15      | As15        |                |             |           | Rakhiyal       | 23.255550°N<br>72.903576°W  |
| 16      | As16        |                |             |           | Babalpura      | 23.214071°N<br>72.864214°W  |
| 17      | As17        |                | Aravalli    | Bayad     | Borol          | 23.238635°N<br>73.219916°W  |
| 18      | As18        |                |             |           | Madhav kampo   | 23.192463°N<br>73.228671°W  |
| 19      | As19        |                |             |           | Demai          | 23.175435°N<br>73.206352°W  |
| 20      | As20        |                |             |           | Bhukhel        | 23.245282°N<br>73.183691°W  |
| 21      | As21        |                |             |           | Bibipura       | 23.267255°N<br>73.218824°W  |
| 22      | As22        |                | Sabarkantha | Prantij   | Dhanpura kampa | 23.238320°N<br>73.081441°W  |
| 23      | As23        |                |             |           | Vadrad         | 23.432129°N<br>72.893863°W  |

| Sr. No. | Isolate No. | Region | District    | Taluka    | Place        | GPS Location               |
|---------|-------------|--------|-------------|-----------|--------------|----------------------------|
| 24      | As24        |        |             |           | Dalpur       | 23.515901°N<br>72.928882°W |
| 25      | As25        |        |             | Bhiloda   | Bhavanath    | 23.787943°N<br>73.244761°W |
| 26      | As26        |        |             | Idar      | Kanpur       | 23.832948°N<br>73.100565°W |
| 27      | As27        |        |             |           | Gorol        | 23.875795°N<br>73.129281°W |
| 28      | As28        |        |             |           | Badoli       | 23.846663°N<br>73.068925°W |
| 29      | As29        |        |             |           | Netramani    | 23.852467°N<br>72.967876°W |
| 30      | As30        |        |             | Vadali    | Chorivad     | 23.929413°N<br>73.125230°W |
| 31      | As31        |        | Banaskantha | Deesa     | Deesa-1      | 24.286991°N<br>72.156044°W |
| 32      | As32        |        |             |           | Aseda        | 24.211109°N<br>72.209240°W |
| 33      | As33        |        |             |           | Deesa-2      | 24.267374°N<br>72.178397°W |
| 34      | As34        |        |             | Dantiwada | Dantiwada    | 24.325896°N<br>72.332206°W |
| 35      | As35        |        |             |           | Nani bhakhar | 24.280839°N<br>72.299247°W |
| 36      | As36        |        |             |           | Vagharol     | 24.085406°N<br>72.244315°W |

The survey was undertaken specifically for pathogen collection and characterization rather than for quantitative estimation of disease prevalence. Fields were selected based on the presence of characteristic early-blight symptoms. Potato leaves showing typical early-blight symptoms were collected and transported to the laboratory. The pathogen was isolated from symptomatic tissue by tissue isolation and purified by the hyphal-tip method. Pure cultures were maintained on PDA slants at 4°C. Isolates were identified on the basis of cultural and morphological characters described by Ellis (1971).

Pathogenicity of *A. solani* isolate As1 was tested on 30-day-old potato plants (cv. Kufri Lauvkar) under glasshouse

conditions. Plants were raised in 30-cm earthen pots containing sterilized soil and farmyard manure (3:1). After surface sterilization with 1% sodium hypochlorite and washing with sterile distilled water, the leaves were spray-inoculated in the evening with a conidial suspension containing  $2 \times 10^6$  conidia mL<sup>-1</sup>. Ten replications and an uninoculated control sprayed with sterile distilled water were maintained. All plants were covered with polyethylene bags for 48 h to maintain high humidity. Disease development was recorded periodically. The pathogen was re-isolated from symptomatic leaves on PDA and compared with the original culture to fulfil Koch's postulates.

## **Cultural and morphological identification of the pathogen**

All 36 isolates were cultured on PDA and Richard's agar and incubated at  $28 \pm 1^\circ\text{C}$ . Colony colour, topography of colony, colony margin, sporulation and hyphal measurement were recorded during 15 days of incubation. Morphological observations were made from 10-day-old cultures. Conidial length and breadth, beak length and breadth and septation were measured under a compound microscope at high magnification. Representative structures were photographed. Identification was based on comparison with the diagnostic description of *A. solani* given by Ellis (1971).

## **Molecular characterization**

### ***Extraction of DNA:***

By using the Cetyl Trimethyl Ammonium Bromide (CTAB) technique, genomic DNA was isolated from 36 pure cultures of *Alternaria* sp. (Fig 1B) for the extraction of the fungal DNA 300 mg fungal mycelium from seven days old culture grown on PDA was ground to a fine powder in liquid nitrogen (LN<sub>2</sub>), using a pre-sterilized mortar and pestle. 1 milliliter of pre-warmed CTAB extraction buffer was added in mortar and pestle and crushed up to sample turned in to liquid. The tubes were incubated in a laboratory water bath at  $65^\circ\text{C}$  for 1 hour with frequent swirling. All of the tubes were filled with an equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged for 15 min at 12,000 rpm at  $4^\circ\text{C}$  to separate the clear phases. The supernatant was precipitated with a double volume of absolute ethanol and mixed properly. The tubes were incubated overnight at  $-20^\circ\text{C}$  to precipitate DNA. After overnight incubation, the sample was centrifuged (ThermoFisher Scientific, Sorvall Legend MicroCL 17R ) at 12,000 rpm for 15

min at  $4^\circ\text{C}$ . The supernatant was discarded and 200  $\mu\text{l}$  of 70 per cent ethanol was added to wash the pellet and centrifuged at 6,000 rpm at  $4^\circ\text{C}$  for 8 minutes. The ethanol was removed completely and the pellet was allowed to air dry on tissue paper for 5 to 10 minutes. The pellet was dissolved into 50  $\mu\text{l}$  of TE buffer. 0.8  $\mu\text{l}$  RNase was added to the dissolved DNA stock and incubated in a water bath at  $37^\circ\text{C}$  for 45 min followed by  $65^\circ\text{C}$  for 10 minutes for enzyme inactivation. The purity of the DNA was determined using 0.8% agarose gels and a Nano-drop (Gel Doc™ XR+ Imaging System (Bio-Rad Laboratories, Hercules, California, USA)).

### ***Primers used:***

**PCR amplification:** PCR was performed with ITS-1 (5'- TCCGTAGGTGAAC CTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATG C-3') (White *et al.*, 1990) as forward and reverse reaction to amplify rDNA-ITS region. Tubes were placed in thermal cycler for 35 cycles of PCR. The program consisted of three distinct steps: Denaturation  $95^\circ\text{C}$  5 min, , annealing  $36-38^\circ\text{C}$  45 sec, extension  $72^\circ\text{C}$  45 sec, final extension  $72^\circ\text{C}$  10 min.

### **Random amplification of polymorphic DNA (RAPD) analysis**

The RAPD primers used for the analysis of random amplification of polymorphic DNA to study the polymorphism present in the isolates of *A. solani*. Total of 20 decamer primers belonging to OPA, OPB, OPC and OPE each series were screened for RAPD analysis Among 80 primers, 5 primers (OPA 11, OPA 12, OPB 9, OPC 6 and OPE 1) were selected based on repeatability. In a 2% agarose gel electrophoresis, the amplified products were seen. A gel documentation system (BioRad, USA) to acquire the digital photos.

## Data scoring

The data were scored based on the presence (1) or absence (0) for analysis. If a product was present in an isolate it was considered as '1' and if absent, was considered as '0'. The data were maintained in the excel sheet format for further analysis. The polymorphism percentage was calculated as per the method suggested by (Blair *et al.*, 1999).

$$\text{Polymorphism (\%)} = \frac{(\text{Total number of bands} - \text{Number of monomorphic bands})}{\text{Total number of bands}} \times 100$$

## Data analysis and dendrogram

The scores were then entered into a matrix for analysis by the numerical taxonomy and multivariate analysis system. The data generated and dendrogram was drawn in NTSYS-pc 1.8 program (Applied Biostatistics Inc., Setauket, NY, USA) (Rohlf, 1993). Clustering was performed using the unweighted pair group method using arithmetic averages (UPGMA).

## RESULTS AND DISCUSSION

### Isolation and pathogenicity

Thirty-six isolates were recovered from potato leaves showing characteristic early-blight symptoms collected from different potato-growing locations in Gujarat. The purified isolates were evaluated for cultural and morphological variability, while isolate As1 was selected as a representative isolate for pathogenicity and ITS-rDNA sequencing.

Spray inoculation of isolate As1 on potato cv. Kufri Lauvkar produced brown, round-to-oval necrotic lesions with characteristic concentric rings 15 days after inoculation, whereas the control plants remained symptomless. The fungus was successfully re-isolated from the inoculated leaves, and the recovered culture corresponded

morphologically with the original culture, thereby fulfilling Koch's postulates and confirming the pathogenicity of isolate As1. Similar development of early-blight lesions following artificial inoculation and successful re-isolation of *A. solani* has been reported in potato and tomato by Nuwamanya *et al.*, (2022) and Abdel-Hamid *et al.* (2023). However, because only As1 was tested, the pathogenicity result applies specifically to this representative isolate and does not establish differences in aggressiveness among all 36 isolates.

### Identification of Pathogen through Internal Transcribed Spacer (ITS) Region

Amplification of the ITS-rDNA region of representative isolate As1 with primers ITS1 and ITS4 produced an amplicon of approximately 540 bp (Fig 1A). A BLAST comparison showed 98–99% sequence identity with the closest *A. solani* reference sequences. The sequence was deposited in GenBank under accession number MZ557830. The ITS result, together with the cultural and morphological characteristics and pathogenicity of As1, supported its identification as *A. solani*.

The ITS region is commonly used for fungal DNA barcoding; however, its ability to distinguish closely related large-spored *Alternaria* species may be limited. Ivanovic *et al.* (2022) demonstrated that potato early-blight symptoms can be associated with *A. solani*, *A. protenta*, *A. grandis* and *A. linariae*, which were differentiated using more informative loci such as GAPDH, calmodulin and RPB2. Therefore, the present ITS result should be regarded as supporting the identification of representative isolate As1 rather than independently confirming every isolate in the collection. Multilocus sequencing of representative isolates from the

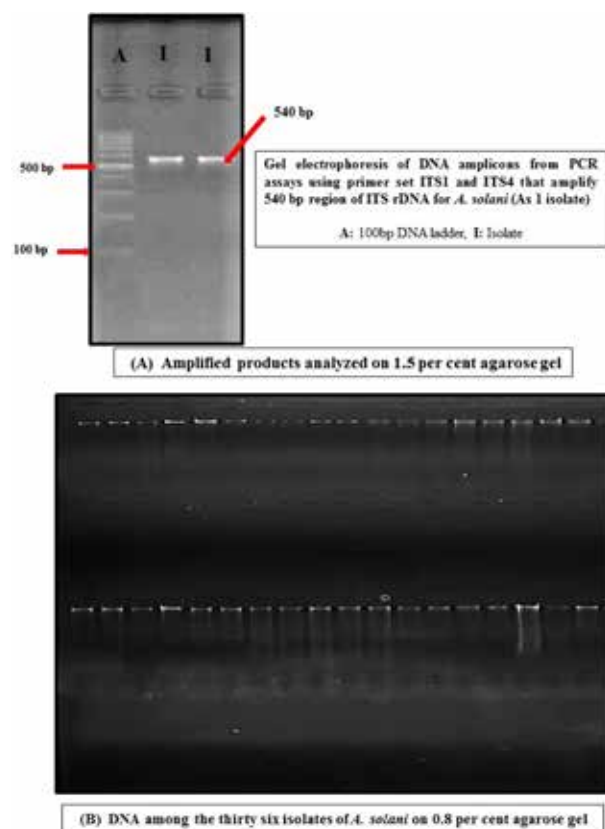


Fig 1A: Amplified product of As-1 isolate

Fig 2A: DNA of thirty-six isolates

cultural and RAPD groups would provide stronger species-level resolution.

### Cultural Characterization of *Alternaria solani*:

#### Potato dextrose agar

Considerable cultural variability was observed among the 36 isolates on PDA. Fluffy growth was predominant (52.78%), followed by aerial (36.11%) and flat growth (11.11%). Smooth, regular margins were most frequent (41.67%), while rough regular and rough irregular margins each accounted for 25.00%. Colony pigmentation was highly variable: the black-grey/grey-to-black group was most frequent (36.11%), followed by greyish-black (19.44%) and dark-black-to-white colonies (16.67%) (Table 2).

Table 2. Frequency distribution of cultural characters of isolates on PDA medium

| A. Colony Topography |                                      |                    |            |
|----------------------|--------------------------------------|--------------------|------------|
| Sr. no               | Topography                           | Number of isolates | Percentage |
| 1                    | Fluffy                               | 19                 | 52.78      |
| 2                    | Aerial                               | 13                 | 36.11      |
| 3                    | Flat                                 | 4                  | 11.11      |
| Total                |                                      | 36                 | 100.00     |
| B. Type of margin    |                                      |                    |            |
| 1                    | Smooth Regular                       | 15                 | 41.67      |
| 2                    | Smooth Irregular                     | 1                  | 2.78       |
| 3                    | Rough Regular                        | 9                  | 25.00      |
| 4                    | Rough Irregular                      | 9                  | 25.00      |
| 5                    | Smooth flat                          | 2                  | 2.56       |
| Total                |                                      | 36                 | 100.00     |
| C. Colony appearance |                                      |                    |            |
| 1                    | Black grey/grey to black             | 13                 | 36.11      |
| 2                    | Greyish black                        | 7                  | 19.44      |
| 3                    | Dark black to white                  | 6                  | 16.67      |
| 4                    | Dark black with white or grey center | 4                  | 11.11      |
| 5                    | Black to white                       | 2                  | 5.56       |
| 6                    | Greenish white                       | 1                  | 2.78       |
| 7                    | Whitish black                        | 1                  | 2.78       |
| 8                    | Dark grey                            | 1                  | 2.78       |
| 9                    | Dark greyish to white                | 1                  | 2.78       |
| Total                |                                      | 36                 | 100.00     |

#### Richard's agar

On Richard's agar, fluffy growth was again predominant (50.00%), followed by flat (36.11%) and aerial growth (13.89%). Smooth, regular margins were most common (41.67%), whereas rough irregular and rough regular margins accounted for 19.44% and 13.89%, respectively. Olive-green colonies and their variants formed the largest pigmentation group (27.78%), followed by dark-black-to-brown/brown variants (25.00%) and green-to-brown/dark-green variants (22.22%) (Table 3).

**Table 3. Frequency distribution of cultural and morphological characters of isolates on Richard's agar medium**

| 1. Cultural characters |                                        |                    |            |
|------------------------|----------------------------------------|--------------------|------------|
| A. Colony Topography   |                                        |                    |            |
| Sr. no                 | Topography                             | Number of isolates | Percentage |
| 1                      | Fluffy                                 | 18                 | 50.00      |
| 2                      | Aerial                                 | 5                  | 13.89      |
| 3                      | Flat                                   | 13                 | 36.11      |
| Total                  |                                        | 36                 | 100.00     |
| B. Type of margin      |                                        |                    |            |
| 1                      | Smooth Regular                         | 15                 | 41.67      |
| 2                      | Smooth Irregular                       | 3                  | 8.33       |
| 3                      | Rough Regular                          | 5                  | 13.89      |
| 4                      | Rough Irregular                        | 7                  | 19.44      |
| 5                      | Regular flat                           | 3                  | 8.33       |
| 6                      | Irregular flat                         | 3                  | 8.33       |
| Total                  |                                        | 36                 | 100.00     |
| C. Colony appearance   |                                        |                    |            |
| 1                      | Olive green and its variants           | 10                 | 27.78      |
| 2                      | Dark black to brown and brown variants | 9                  | 25.00      |
| 3                      | Green to brown and dark green variants | 8                  | 22.22      |
| 4                      | Black to grey and grey variants        | 5                  | 13.89      |
| 5                      | Pale cream with olive-green margin     | 2                  | 5.56       |
| 6                      | Dark brown                             | 2                  | 5.56       |
| Total                  |                                        | 36                 | 100.00     |

Differences in the frequencies of growth patterns and colony pigmentation between the two media indicate that cultural expression was influenced by both isolate genotype and medium composition. The predominance of fluffy colonies was similar on both media, but aerial growth was more frequent on PDA, whereas flat growth was more frequent on Richard's agar. Comparable isolate-dependent variation in colony colour, growth pattern, margin and pigmentation has been reported among *A. solani* isolates by Mugao *et al.* (2021) and Nuwamanya *et al.* (2022). These studies also observed substantial phenotypic heterogeneity among

isolates collected from different locations. Consequently, colony characteristics are useful for describing variability within an isolate collection but should not be used alone for definitive species identification.

### Morphological Characterization of *Alternaria solani*:

Marked variation was recorded in conidial and beak dimensions among the isolates (Table 4). On PDA, conidial length ranged from 12.5 to 89.4  $\mu\text{m}$  and conidial breadth from 4.6 to 30.5  $\mu\text{m}$ . Beak length ranged from 4.9 to 43.4  $\mu\text{m}$ , while beak breadth ranged from 0.6 to 9.5  $\mu\text{m}$ . Isolate As25 exhibited the widest conidial-length range of 24.8–89.4  $\mu\text{m}$ , whereas As12 included the lowest recorded conidial length, with a range of 12.5–51.8  $\mu\text{m}$ .

Conidia produced on PDA contained up to three longitudinal and seven transverse septa. A longitudinal-septation range of 0–2 was most frequent and occurred in 17 isolates, whereas a transverse-septation range of 2–4 occurred in 16 isolates. Isolates As13 and As18 produced conidia with up to three longitudinal septa, while As18 exhibited the maximum of seven transverse septa.

On Richard's agar, conidial length ranged from 12.1 to 82.4  $\mu\text{m}$  and breadth from 4.1 to 27.7  $\mu\text{m}$ . Beak length ranged from 4.6 to 37.0  $\mu\text{m}$ , while beak breadth ranged from 0.4 to 9.1  $\mu\text{m}$ . Conidia produced on this

**Table 4: Morphological characters of isolates on Potato Dextrose Agar and Richard's agar medium**

| Sr. no. | Character                          | PDA (Range) | Richard's agar (Range) |
|---------|------------------------------------|-------------|------------------------|
| 1       | Conidial length ( $\mu\text{m}$ )  | 12.5–89.4   | 12.1–82.4              |
| 2       | Conidial breadth ( $\mu\text{m}$ ) | 4.6–30.5    | 4.1–27.7               |
| 3       | Beak length ( $\mu\text{m}$ )      | 4.9–43.4    | 4.6–37.0               |
| 4       | Beak breadth ( $\mu\text{m}$ )     | 0.6–9.5     | 0.4–9.1                |
| 5       | Vertical septa                     | 0–3         | 0–4                    |
| 6       | Horizontal septa                   | 2–7         | 0–7                    |

medium contained up to four longitudinal and seven transverse septa. A longitudinal-septation range of 0–2 was observed most frequently, occurring in 19 isolates, whereas a transverse-septation range of 1–4 occurred in 12 isolates. Isolates As1 and As11 produced conidia with up to four longitudinal septa, while As1 showed the maximum of seven transverse septa.

Despite their quantitative variation, the isolates produced pigmented, muriform and beaked conidia consistent with the morphological description of large-spored *Alternaria*. Variation in conidial dimensions, septation and beak size among isolates grown under standardized conditions suggests substantial phenotypic heterogeneity within the Gujarat collection. Yadav and Pathak (2011), Marak *et al.* (2014), Pachori *et al.* (2016), Kaur *et al.* (2020), Mugao *et al.* (2021) and Nuwamanya *et al.* (2022) similarly reported significant isolate-to-isolate variation in conidial shape, dimensions and septation among *A. solani* isolates. The differences observed between PDA and Richard's agar in the present investigation also demonstrate that the culture medium can influence the expression of diagnostic morphological characteristics. Morphological measurements should therefore be interpreted in combination with pathogenicity and molecular evidence.

### Molecular Characterization of *Alternaria solani* with the use of RAPD primers:

Of the 80 RAPD primers initially screened, five primers—OPA-11, OPA-12, OPB-09, OPC-06 and OPE-01—generated clear and reproducible amplification profiles and were selected for analysis of the 36 isolates. Together, these primers produced 30 scorable loci, of which 28 were polymorphic, corresponding to an overall polymorphism of 93.3% (Table 5). The number of loci amplified per primer ranged from five to seven. OPA-11, OPC-06 and OPE-01 showed 100% polymorphism, whereas OPA-12 and OPB-09 showed 83.3 and 85.7% polymorphism, respectively. OPA-11 exhibited the highest polymorphic information content of 0.44 and the highest resolving power of 4.22, indicating that it was the most informative of the five primers for differentiating the isolates (Fig 3).

The high proportion of polymorphic loci indicates considerable RAPD-based genetic variability among the Gujarat isolates. Molecular heterogeneity among *A. solani* isolates has also been reported using RAPD, AFLP, microsatellite and sequence-based markers. Abdel-Hamid *et al.* (2023) demonstrated RAPD-based differentiation among potato isolates of *A. solani* from different Egyptian locations. Adhikari *et al.* (2020) detected high nucleotide diversity and

**Table 5: RAPD marker characteristics, polymorphism and informativeness among 36 *A. solani* isolates**

| Sr. No. | Primers | Primer sequence (5'-3') | TB  | TL | NPL | PP    | PIC  | Rp    | MI   |
|---------|---------|-------------------------|-----|----|-----|-------|------|-------|------|
| 1       | OPA 11  | CAATCGCCGT              | 90  | 6  | 6   | 100.0 | 0.44 | 4.22  | 2.66 |
| 2       | OPA 12  | TCGGCGATAG              | 116 | 6  | 5   | 83.3  | 0.29 | 2.22  | 1.43 |
| 3       | OPB 9   | TGGGGGACTC              | 132 | 7  | 6   | 85.7  | 0.30 | 2.89  | 1.81 |
| 4       | OPC 6   | GAACGGACTC              | 102 | 6  | 6   | 100.0 | 0.39 | 3.44  | 2.33 |
| 5       | OPE 1   | CCCAAGGTCC              | 38  | 5  | 5   | 100.0 | 0.31 | 2.11  | 1.54 |
|         |         | Total                   | 478 | 30 | 28  | 93.3  | 1.73 | 14.88 | -    |

TB: total number of bands amplified across 36 isolates, TL: Total number of loci, NPL: number of polymorphic loci, PP: percentage polymorphism, PIC: polymorphic information content, Rp: resolving power, MI: marker index

admixed haplotypes among *Alternaria* species associated with potato and tomato, while Adhikari *et al.* (2021) reported high genotypic diversity within *A. solani* and related *Alternaria* populations. Collectively, these findings indicate that considerable genetic diversity can occur within geographically sampled *Alternaria* populations.

Clustering and PCoA

UPGMA analysis separated the 36 isolates into two major clusters, A and B. Cluster A contained nine isolates, including the distinct placement of As8, whereas cluster B contained the remaining 27 isolates distributed among several subclusters (Fig. 2) The clustering pattern

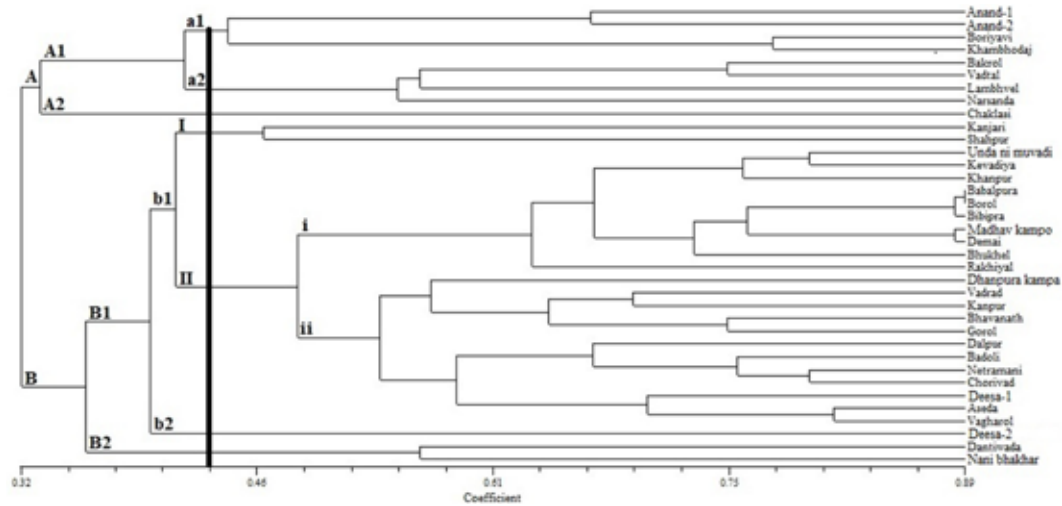
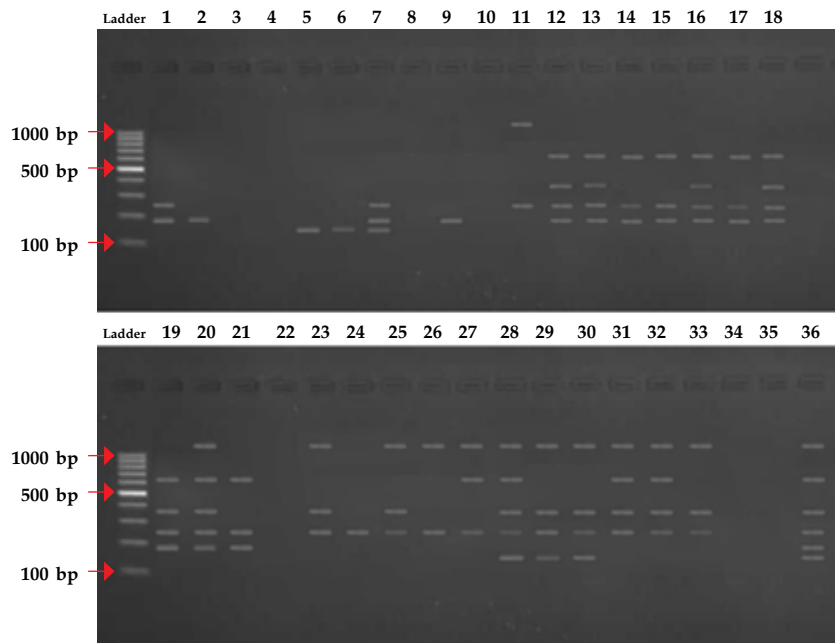


Fig 2: RAPD UPGMA dendrogram of thirty-six isolates of *A. solani* based on Jaccard's similarity coefficient



OPA-11 : RAPD profile of primer

Fig 3: RAPD profile of OPA-11 primer

was not strictly associated with district of collection because isolates originating from different districts frequently occurred within the same subcluster. Conversely, isolates from geographically separated locations occasionally showed high genetic similarity. The complete placement of individual isolates is presented in the dendrogram rather than repeated in the text.

The PCoA supported the broad differentiation observed by UPGMA. The first and second principal coordinates explained 21.65 and 18.59% of the variation, respectively, and together accounted for 40.24% of the total RAPD variation. The third coordinate explained a further 8.99%. Similar weak associations between molecular clustering and geographical origin were observed by Nuwamanya *et al.* (2022) and Adhikari *et al.* (2021). Abdel-Hamid *et al.* (2023) also found that the molecular relationships among *A. solani* isolates were not determined solely by their geographical distribution.

### Population diversity and AMOVA

The North Gujarat population showed greater RAPD-based diversity than the Middle Gujarat population. North Gujarat

had higher values for the observed number of alleles or band states ( $N_a = 1.93 \pm 0.04$ ), effective number of alleles ( $N_e = 1.50 \pm 0.05$ ), Shannon's information index ( $I = 0.48 \pm 0.03$ ), expected heterozygosity ( $H_e = 0.32 \pm 0.03$ ) and percentage of polymorphic loci (93.33%). Six private RAPD bands were recorded in North Gujarat, whereas no private bands were detected in Middle Gujarat (Table 6). Part of this difference may be attributable to the larger number of isolates sampled from North Gujarat; consequently, direct comparison of diversity estimates between the two populations should be interpreted cautiously.

Nei's unbiased genetic distance between the Middle and North Gujarat populations was low ( $D = 0.064$ ), while their genetic identity was high (0.938). AMOVA attributed 80% of the total molecular variation to differences within populations and 20% to differences between populations, with  $\Phi_{PT} = 0.198$  (Table 7). Thus, most genetic variation was distributed within the two geographical populations rather than between them. Adhikari *et al.*, (2021) similarly found that more than 85% of the genetic variation in *Alternaria* populations from potato and

**Table 6: Genetic diversity estimates for different geographic populations of *A. solani* across Gujarat.**

| Pop            | N     | $N_a$          | $N_e$           | I               | $H_e$            | uHe             | PAL | %P    |
|----------------|-------|----------------|-----------------|-----------------|------------------|-----------------|-----|-------|
| Middle Gujarat | 13.0  | $1.5 \pm 0.15$ | $1.30 \pm 0.06$ | $0.30 \pm 0.05$ | $0.190 \pm 0.03$ | $0.20 \pm 0.03$ | 0   | 73.33 |
| North Gujarat  | 23.0  | $1.9 \pm 0.04$ | $1.50 \pm 0.05$ | $0.48 \pm 0.03$ | $0.32 \pm 0.03$  | $0.32 \pm 0.03$ | 6   | 93.33 |
| Mean           | 36.0* | $1.7 \pm 0.08$ | $1.4 \pm 0.04$  | $0.39 \pm 0.03$ | $0.25 \pm 0.02$  | $0.26 \pm 0.02$ | 6   | 83.33 |

N = number of individuals,  $N_a$  = number of different alleles,  $N_e$  = number of effective alleles, I = Shannon information index,  $H_e$  = expected heterozygosity, uHe = unbiased expected, heterozygosity, PAL = private alleles, % P = percent polymorphism among population. Analyses were performed at a 95% confidence interval using GenAlEx version 6.5 software.

**Table 7: Analysis of molecular variance (AMOVA) among and within different geographic populations of *A. solani*.**

| Source of variation | df | SS     | MS    | Variance component | Variation (%) |
|---------------------|----|--------|-------|--------------------|---------------|
| Among populations   | 1  | 24.35  | 24.35 | 1.18               | 20            |
| Within populations  | 34 | 161.98 | 4.76  | 4.76               | 80            |
| Total               | 35 | 186.33 | –     | 5.94               | 100           |

df, degrees of freedom; SS, sum of squares; MS, mean squares; VC, variance component; Var (%), percentage of total molecular variation.

tomato occurred within populations, with limited population subdivision according to geographical location.

The high genetic identity between the two Gujarat populations, the predominance of within-population variation and the occurrence of isolates from different districts in the same UPGMA subclusters indicate only partial geographical structuring. Movement of infected planting material and dispersal of airborne conidia could contribute to the distribution of related genotypes across potato-growing areas, although these mechanisms were not directly investigated. The observed diversity nevertheless emphasizes the importance of including isolates from multiple locations when screening potato germplasm for early-blight resistance or evaluating disease-management practices.

RAPD is a dominant marker system and can be sensitive to amplification conditions. Therefore, the clusters obtained in the present study represent RAPD-based phenetic relationships and should not be interpreted as definitive phylogenetic lineages. Furthermore, the isolates were collected during a single season, pathogenicity was assessed only for representative isolate As1, and only As1 was subjected to ITS sequencing. Future investigations should combine multiyear sampling with pathogenicity, fungicide-sensitivity testing and multilocus or genome-wide sequencing to clarify the biological and geographical significance of the variability observed among Gujarat isolates.

## CONCLUSIONS

The study revealed substantial cultural, morphological and RAPD-based genetic variability among 36 *A. solani* isolates collected from major potato-growing regions of Gujarat. ITS-rDNA sequencing

of representative isolate As1 supported its species identification, while RAPD analysis demonstrated high polymorphism and measurable population structure across the isolate collection. Most molecular variation occurred within rather than between the two predefined geographic populations. The observed diversity emphasizes the need to consider pathogen variability in early-blight surveillance, resistance breeding and integrated disease-management programmes. The single-season sampling, sequencing of only one representative isolate and use of dominant RAPD markers limit taxonomic and population-genetic inference; broader multi-season sampling with multilocus or genome-scale sequencing is recommended.

## AUTHOR CONTRIBUTIONS

JVP: methodology, investigation, data curation, writing—original draft. NMG: conceptualization, supervision, data curation. NBP: validation, writing—review and editing. TCB: software and data analysis. All authors reviewed and approved the manuscript.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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