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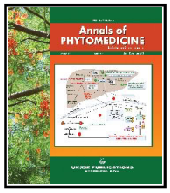

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Molecular characterization of *Alternaria burnsii* causing blight of cumin using random amplified polymorphic DNA (RAPD) markers

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Abstract

Ten isolates of the cumin blight pathogen (*Alternaria burnsii*) were obtained from the major cumin-growing regions of Rajasthan state in India during the Rabi seasons of 2019-20 and 2020-21. The isolates were molecularly characterized using genomic DNA and random amplified polymorphic DNA (RAPD) analysis with five decamer primers from the OPA and OPE series. A total of ten isolates were chosen from the major cumin-growing areas of Rajasthan, and out of the ten RAPD primers used initially, only five were chosen based on their repeatability. The RAPD analysis showed 100% polymorphism, indicating genetic diversity among the isolates. Dendrogram analysis was performed to group the isolates based on their genetic relatedness, resulting in two major clusters designated C1 and C2. Cluster C1 contained nine sub-clusters, including Ab-RSR, Ab-OSN, Ab-BLR, Ab-BHML, Ab-RNIW, Ab-KKR, Ab-KSG, Ab-NKH, and Ab-BCWL, while cluster C2 had only one isolate, Ab-BLTR. The similarity matrix showed that Ab-BCWL had a higher similarity coefficient value of 1.000, indicating greater genetic similarity among the isolates in this sub-cluster, while Ab-BLTR had a lower similarity coefficient value of 0.222, indicating less genetic similarity with the other isolates. The similarity coefficient represents the degree of genetic similarity between the isolates, and its variation among the isolates provides insight into the genetic diversity of the cumin blight pathogen population in Rajasthan. The genetic diversity analysis provides crucial insights into the pathogen's population structure. This understanding of genetic clusters and sub-clusters will be essential for recognizing different virulent strains or variations that could influence disease severity and resistance patterns, ultimately leading to the development of more effective management strategies for cumin blight.

1. Introduction

Spices are the fundamental components of flavour in food. One of the major purposes of spices is to give food aroma, texture, and colour. They also serve as a preservative and offer nutritional and health advantages. Cumin (*Cuminum cyminum* L.) is a highly valued seed spice and holds the distinction of being one of the earliest and most significant spices known to humankind. In the present time, cumin is being grown all over the world by humankind. However, while it is now grown all over the world for its pleasingly aromatic seed, it is believed that it is native to Egypt and Syria, Eastern Mediterranean and Turkestan region.

Diseases that significantly impact the quantity and quality of cumin cultivation pose ongoing challenges for farmers. These diseases have a detrimental effect on the crop's overall quality, often resulting in lower market prices (Rex *et al.,* 2019). Among the various diseases affecting cumin, wilt, blight, and powdery mildew are particularly noteworthy (Dange, 1995).

In subtropical countries, Alternaria blight is characterized as the most severe cumin disease. This disease is quite common and damaging since it damages all parts of the plant that are above ground, including the seed, thus reducing crop yield. Depending on the environmental conditions, *A. burnsii* only damages the cumin plant after the flowering stage and in some years completely destroys the yield according to Uppal *et al.* (1938) and Gemawat and Prasad (1971).

Determining variability amongst *Alternaria* species based on molecular approaches is essential. DNA markers have emerged into an effective tool for investigating the molecular genetics and taxonomy of many organisms. Singh *et al.* (2016) highlighted the utility of RAPD based analysis in evaluating genetic relatedness within fungal species. The technique does not require prior knowledge of the DNA sequence, making it a valuable tool for evaluating genetic relatedness and diversity within organisms, including fungi.

Comprehensive understanding of molecular variability is crucial for developing effective breeding programs for disease-resistant cumin varieties. Indeed, traditional methods of identifying plant

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parasitic fungi through cultural, morphological, and conidial traits have been valuable for many years in distinguishing different fungal species (Tupke *et al.,* 2021). These methods are often based on observable characteristics such as growth patterns, colony morphology, and reproductive structures (conidia). However, as Sawant *et al.* (2022) pointed out, these traditional approaches have some limitations when it comes to providing a comprehensive understanding of the genetic variation and systematic relationships among closely related fungal isolates. Furthermore, disease development rates can impact the variants or isolates within the population of the phytopathogen. Therefore, molecular techniques like random amplified polymorphic DNA (RAPD) markers are essential for investigating the genetic diversity of *A. burnsii* isolates collected from various geographical regions of Rajasthan. Genetic similarity between the isolates, and its variation among the isolates provides insight into the genetic diversity of the cumin blight pathogen population in Rajasthan. Thus, this study was aimed to investigate the genetic similarity and variation among *A. burnsii* isolates using RAPD markers.

2. Materials and Methods

The investigation involved the isolation of ten isolates of *A.burnsii* obtained from the Department of Plant Pathology, SKRAU, Bikaner. The research procedures and protocols were conducted at SP Institute of Biotechnology, Jaipur, following established methodologies. These isolates were obtained from different locations of the surveyed district of Rajasthan and have been mentioned in Table 1. Using the RAPD technique, DNA isolated from several isolates was compared.

Table 1: Details of an isolate obtained from different locations

Location of collection		Isolate designation
District	Village/Town	
Barmer	Ramsar	Ab-RSR
Barmer	Balotra	Ab-BLTR
Jodhpur	Osian	Ab-OSN
Jodhpur	Bilara	Ab-BLR
Jalore	Bhinmal	Ab-BHML
Jalore	Raniwara	Ab-RNIW
Ajmer	Kekri	Ab-KKR
Ajmer	Kishangarh	Ab-KSG
Bikaner	Nokha	Ab-NKH
Bikaner	Beechwal	Ab-BCWL

2.1 Genomic DNA extraction

The collection of fungal mycelia from each isolate was performed, and it was then transferred to a sterile microcentrifuge tube. To preserve the DNA, the mycelium was rapidly frozen in liquid nitrogen and subsequently processed using the CTAB (cetyl trimethyl ammonium bromide) method as outlined by Doyle and Doyle (1987) with slight modifications.

2.2 RAPD primers selection

To analyze the variability in the extracted genomic DNA, PCR amplification was initially performed using a total of ten different primers. After an initial screening process, five primer sets (OPA-13, OPA-9, OPE-4, OPE-7, and OPA-18) were carefully selected based on their repeatability for the RAPD analysis. The details of the specific RAPD primers used to investigate polymorphism in *A. burnsii* isolates are provided in the following Table 2.

Table 2: Selected RAPD primers for polymorphism analysis

S.No.	Primer	Sequence (5'-3')	Length of primer (bp)
1	OPA-13	CAG CAC CCA C	10
2	OPA-9	GGG TAA CGC C	10
3	OPE-4	GTG ACA TGC C	10
4	OPE-7	AGA TGC AGC A	10
5	OPA-18	AGG TGA CCG T	10

A reaction mixture of 20 µl was prepared using 1x assay buffer. Taq DNA polymerase (1.5 units/µl) was added to the reaction mixture. Deoxynucleotide triphosphates (dNTPs were added at a concentration of 200 µM for each dNTP. Random primers (operon technologies) were added at a concentration of ten pmol per reaction. Template DNA was included at a concentration of 100 ng. The PCR reaction was conducted using a Biometra Thermocycler under standard cycling conditions. The specific cycling parameters, including denaturation, annealing, and extension steps, were optimized based on the requirements of the RAPD assay and the nature of the template DNA. After PCR amplification, the resulting products were resolved and separated using 1.2% agarose gel in 1X TBE buffer *via* electrophoresis at 100 V. The separated DNA fragments were visualized by staining with ethidium bromide and observed under a UV transilluminator. The banding patterns obtained from the electrophoresis allowed for the analysis of polymorphisms in the *A. burnsii* isolates using the selected RAPD primers listed in Table 2.

2.3 Banding pattern evaluation of amplified DNA fragments

The banding patterns of amplified DNA fragments using the RAPD technique were carefully analyzed. Each band observed in the gel represented a distinct DNA fragment that had undergone amplification during the PCR reaction. To distinguish between these bands, their molecular sizes were determined, and the 100 bp ladder served as a reference for calibrating the molecular weights of the PCR products obtained with different primers.

For analysis, data were evaluated according to their existence (1) or absence (0). A product was assigned a rating of 1, if it was present in a genotype, and a rating of 0 if it was not present. Faintly visible bands were not individually scored. However, if there was a major band that corresponded to a faint band, it was taken into account and scored accordingly. All of the primers were duplicated, if necessary to confirm the existence of bands and assess the repeatability. For subsequent examination, the information was retained in an excel sheet format. The Blair and Sangwan (1999) technique were used to compute the polymorphism percentage.

Polymorphism (%) =

$$\left(\frac{\text{Total number of bands} - \text{Number of monomorphic bands}}{\text{Total number bands}}\right) \times 100$$

2.4 Data analysis

The UPGMA (unweighted pair group method with arithmetic mean) procedure was used to examine the RAPD-generated data. Initially, a distance matrix was calculated, establishing pairwise genetic dissimilarities between the isolates. Each isolate was treated as an individual cluster, and the two clusters with the smallest genetic distance were iteratively identified. These clusters were merged into composite clusters, and the distances from these new clusters to the remaining clusters were updated based on the average of the individual cluster distances. This process continued until a hierarchical dendrogram was formed, depicting the relationships between the isolates. The UPGMA algorithm assumed a constant evolutionary rate and provided insights into genetic relatedness based on the average distances at which clusters were merged.

2.5 Dendrogram construction

A dendrogram was constructed using the UPGMA clustering method, which was implemented using the NTSYS pc version 2.02 computer software. The dendrogram was created using the genetic distances determined by cluster analysis through UPGMA, enabling the visualization of links between the isolates.

3. Results

3.1 Spectrophotometric analysis and purity assessment of DNA from *A. burnsii* isolates

The CTAB method was employed for the extraction of whole genomic DNA from *A. burnsii* isolates. Subsequently, an RNase treatment was performed to enzymatically degrade any residual RNA contaminants. The concentration of DNA was determined using the UV absorbance technique. Spectrophotometric analysis revealed the DNA concentrations in the mycelia of the isolates to be as follows: Ab-RSR (534 ng/µl), Ab-BLTR (340 ng/µl), Ab-OSN (349 ng/µl), Ab-BLR (250 ng/µl), Ab-BHML (320 ng/µl), Ab-RNIW (220 ng/µl), Ab-KKR (340 ng/µl), Ab-KSG (210 ng/µl), Ab-NKH (225 ng/µl), and Ab-BCWL (213 ng/µl). Furthermore, the absorbance ratio of DNA at the 260/280 nm wavelength was measured and found to be 1.82, 1.79, 1.81, 1.83, 1.80, 1.83, 1.85, 1.80, 1.87, and 1.81 for the respective isolates (Table 3). DNA samples exhibiting A260/A280 ratios ranging from 1.9 to 2.0 are considered indicative of high purity. all the extracted DNA samples displayed ratios between 1.79 and 1.87, thus confirming their suitability for subsequent RAPD (Random Amplified Polymorphic DNA) analysis. The isolated DNA exhibited a single, distinct, and sharp band on agarose gel electrophoresis, confirming the presence of high molecular weight DNA.

Table 3: DNA concentrations and absorbance ratios in *A. burnsii* isolates

Isolates	Location	Absorbance ratio(260/280)	Concentration (ng/µl)
Ab-RSR	Ramsar	1.82	534
Ab-BLTR	Balotra	1.79	340
Ab-OSN	Osian	1.81	349
Ab-BLR	Bilara	1.83	250
Ab-BHML	Bhinmal	1.80	320
Ab-RNIW	Raniwara	1.83	220
Ab-KKR	Kekri	1.85	340
Ab-KSG	Kishangarh	1.80	210
Ab-NKH	Nokha	1.87	225
Ab-BCWL	Beechwal	1.81	213

3.2 Molecular variability and polymorphism analysis using RAPD analysis in *A. burnsii* isolates

A total of ten isolates, *i.e.,* Ab-BCWL, Ab-NKH, Ab-RSR, Ab-BLTR, Ab-BHML, Ab-RNIW, Ab-KKR, Ab-KSG, Ab-OSN and Ab-BLR of *A. burnsii* were used to determined molecular variability. Under Figures (1-5), the outcomes that resulted from employing five primers are displayed. The Table (4) provides details of genetic diversity and polymorphism analysis for *A. burnsii* isolates using five RAPD primers (OPA-13, OPA-9, OPE-4, OPE-7, and OPA-18). The primer sequences amplified DNA fragments of specific allele sizes, and the number of loci and polymorphic loci were determined for each primer. The overall polymorphism percentage was calculated based on the number of polymorphic loci. The total number of bands observed for each primer is also provided. For all five primers, a dendrogram constructed by the UPGMA programme has been presented.

OPA-13 primer amplified DNA fragments ranging from 120 to 700 base pairs (bp) (Figure 1 and Table 4). For the OPA-13 primer, 9 out of 10 isolates showed polymorphism, resulting in a polymorphism percentage of 90%. A total of 82 bands were observed.

OPA-9 primer generated DNA fragments ranging from 400 to 700 bp. It targeted 4 loci, with 2 of them showing polymorphism, leading to a polymorphism percentage of 50% (Figure 2 and Table 4). A total of 19 bands were observed.

OPE-4 primer amplified DNA fragments ranging from 160 to 800 bp (Figure 3 and Table 4). It detected 9 loci, all of which were polymorphic, resulting in a polymorphism percentage of 100%. A total of 48 bands were observed.

OPE-7 primer generated DNA fragments ranging from 300 to 800 bp. It targeted 6 loci, out of which 5 showed polymorphism, resulting in a polymorphism percentage of 83.33%. A total of 85 bands were observed (Figure 4 and Table 4).

OPA-18 primer amplified DNA fragments ranging from 200 to 500 bp (Figure 5 and Table 4). It detected 4 loci, all of which were polymorphic, resulting in a polymorphism percentage of 100%. A total of 58 bands were observed.

Table 4: DNA banding profile and polymorphism analysis of 5 RAPD primers

S.No.	Primer	Allele size (bp)	No. of loci	No. of polymorphic loci	Polymorphism %	Total bands
1	OPA-13	120-700	10	9	90	82
2	OPA-9	400-700	4	2	50	19
3	OPE-4	160-800	9	9	100	48
4	OPE-7	300-800	6	5	83.33	85
5	OPA-18	200-500	4	4	100	58
		Total	33	29	423.33	292
		Mean	6.6	5.8	84.66	58.4

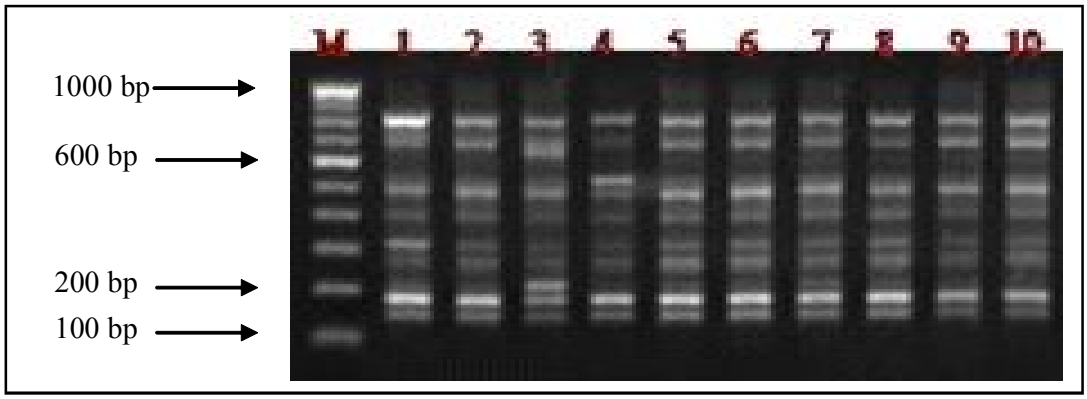

Figure 1: RAPD profile of OPA-13 primer.
1-Ab-RSR; 2-Ab-BLTR; 3-Ab-OSN; 4-Ab-BLR; 5-Ab-BHML; 6-Ab-RNIW; 7-Ab-KKR; 8-Ab-KSG; 9-Ab-NKH; 10-Ab-BCWL; M-100 bp DNA ladder

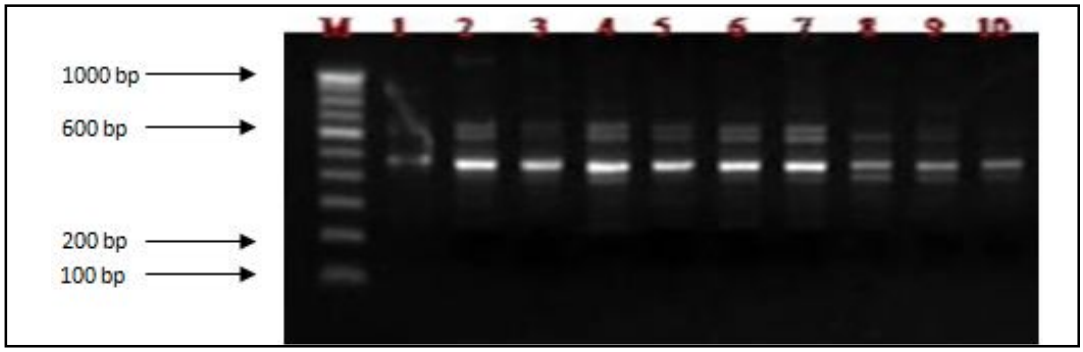

Figure 2: RAPD profile of OPA-9 primer.
1- Ab-RSR; 2-Ab-BLTR; 3-Ab-OSN; 4-Ab-BLR; 5-Ab-BHML; 6-Ab-RNIW; 7-Ab-KKR; 8-Ab-KSG; 9-Ab-NKH; 10-Ab-BCWL

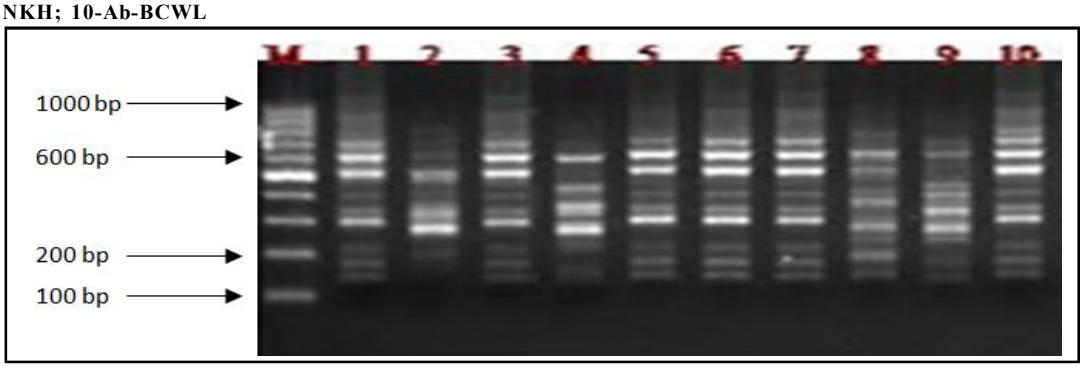

Figure 3: RAPD profile of OPE-4 primer.
1- Ab-RSR; 2-Ab-BLTR; 3-Ab-OSN; 4-Ab-BLR; 5-Ab-BHML; 6-Ab-RNIW; 7-Ab-KKR; 8-Ab-KSG; 9-Ab-NKH; 10-Ab-BCWL

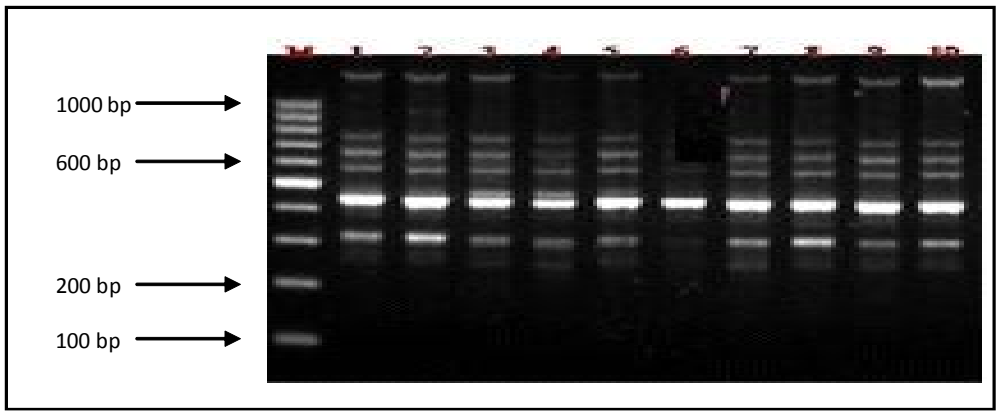

Figure 4: RAPD profile of OPE-7 primer.
1- Ab-RSR; 2-Ab-BLTR; 3-Ab-OSN; 4-Ab-BLR; 5-Ab-BHML; 6-Ab-RNIW; 7-Ab-KKR; 8-Ab-KSG; 9 Ab-NKH; 10-Ab-BCWL

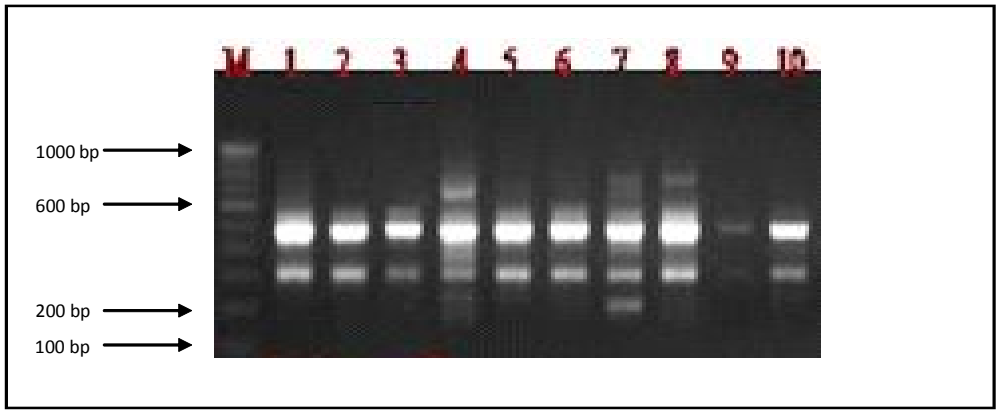

Figure 5: RAPD profile of OPA-18 primer.
1- Ab-RSR; 2-Ab-BLTR; 3-Ab-OSN; 4-Ab-BLR; 5-Ab-BHML; 6-Ab-RNIW; 7-Ab-KKR; 8-Ab-KSG; 9-Ab-NKH; 10-Ab-BCWL

3.3 Polymorphism detected by RAPD primers

Genetic diversity was studied among ten isolates using 5 RAPD primers of OPA and OPE Series. Data presented in Table 4 revealed that polymorphism per cent for five primers ranged from 50 to 100. The number of loci per primer varied from 4 to 10 and the number of polymorphic loci varied from 2 to 9. Primer OPA-13 produced ten loci and polymorphism was 90%. Primer OPA-9 produced four loci and polymorphism was 50. Primer OPE-4 produced 9 loci and 9 polymorphic loci and polymorphism was 100. Primer OPE-7 produced 6 loci and 5 polymorphic loci and polymorphism was 83.33. Primer OPA-18 produced 4 loci and 4 polymorphic loci and polymorphism was 100.

3.4 Similarity matrix analysis

The genetic similarity matrix based on Jaccard's similarity coefficient was constructed to assess the genetic relationships among the isolates of *A. burnsii*. This analysis provides valuable insights into the genetic variability and relatedness of the studied isolates. The matrix, presented in Table 5, reveals the pairwise genetic similarity coefficients between the *ten A. burnsii* isolates, namely Ab-RSR, Ab-BLTR, Ab-OSN, Ab-BLR, Ab-BHML, Ab-RNIW, Ab-KKR, Ab-KSG, Ab-NKH, and Ab-BCWL.

Upon examining the matrix, several observations can be made. Firstly, the diagonal elements of the matrix indicate a similarity coefficient of 1.000, reflecting the identical nature of each isolate when compared to itself. Moving to the off-diagonal elements, which represent the genetic similarities between pairs of isolates, different levels of genetic relatedness are observed.

For instance, the comparison between Ab-RSR and Ab-BLTR demonstrates a similarity coefficient of 0.750, indicating a moderate level of genetic similarity. Similarly, the pairings of Ab-KSG and Ab-OSN, Ab-NKH and Ab-BCWL, Ab-KKR and Ab-BLTR, and Ab-NKH and Ab-BLTR all exhibit similarity coefficients of 0.750, indicating substantial genetic relatedness. Conversely, comparisons such as Ab-RNIW and Ab-BCWL yield a lower similarity coefficient of 0.300, suggesting a more distant genetic relationship.

The symmetric nature of the matrix reinforces the bidirectional genetic similarity between isolates. Moreover, the coefficients ranging from 0 to 1 further emphasize the variability in genetic relatedness among the isolates. Higher coefficients indicate greater genetic similarity, while lower coefficients reflect greater genetic divergence.

Table 5: Similarity matrix among *A. burnsii* isolates using Jaccard's similarity coefficients

Isolate	Ab-RSR	Ab-BLTR	Ab-OSN	Ab-BLR	Ab-BHML	Ab-RNIW	Ab-KKR	Ab-KSG	Ab-NKH	Ab-BCWL
Ab-RSR	1.000									
Ab-BLTR	0.750	1.000								
Ab-OSN	0.666	0.857	1.000							
Ab-BLR	0.888	0.666	0.600	1.000						
Ab-BHML	0.888	0.666	0.600	0.800	1.000					
Ab-RNIW	0.300	0.375	0.333	0.273	0.273	1.000				
Ab-KKR	0.750	1.000	0.857	0.666	0.666	0.375	1.000			
Ab-KSG	1.000	0.750	0.666	0.888	0.888	0.300	0.750	1.000		
Ab-NKH	1.000	0.750	0.666	0.888	0.888	0.300	0.750	1.000	1.000	
Ab-BCWL	0.750	0.222	0.444	0.666	0.666	0.622	0.500	0.750	0.750	1.000

3.5 Dendrogram depicting genetic links among *A. burnsii* isolates

The dendrogram in Figure 6 illustrates the genetic relationships among different *A. burnsii* isolates based on RAPD banding patterns. Cluster analysis using the UPGMA method was performed, and the resulting dendrogram grouped the isolates into two major clusters, C1 and C2. Cluster C1 consists of nine subclusters, while C2 contains a single isolate, Ab-BLTR.

Cluster C1 is further divided into C1A and C1B. C1A is also divided into two subclusters, C1A a and C1A b. C1A a is subsequently divided into C1A aa and C1A ab. Specifically, the C1A aa subcluster includes the isolates Ab-BCWL, Ab-NKH, and Ab-RSR, while the C1Aab subcluster includes Ab-BHML and Ab-RNIW. Cluster C1Ab includes Ab-OSN and Ab-BLR. Lastly, Cluster C1B comprises the isolates Ab-KKR and Ab-KSG.

The dendrogram reveals that Ab-BCWL shares a higher similarity coefficient with Ab-NKH and Ab-RSR, indicating a closer genetic relationship between these isolates. On the other hand, Ab-BLTR exhibits a lower similarity coefficient with the other isolates. The similarity matrix analysis indicated that the isolates exhibited similarity coefficients ranging from 65% to 100%.

The UPGMA-generated dendrogram divided the *A. burnsii* isolates into two main clusters, with Cluster C1 containing the majority of isolates and Cluster C2 consisting of a single isolate, Ab-BLTR. Notably, Ab-BCWL demonstrated the highest similarity coefficients with Ab-NKH (0.94) and Ab-RSR (0.89), while Ab-BLTR exhibited the lowest similarity coefficient (0.65). These results highlight the presence of polymorphism, with similarity coefficients ranging from 50% to 100%.

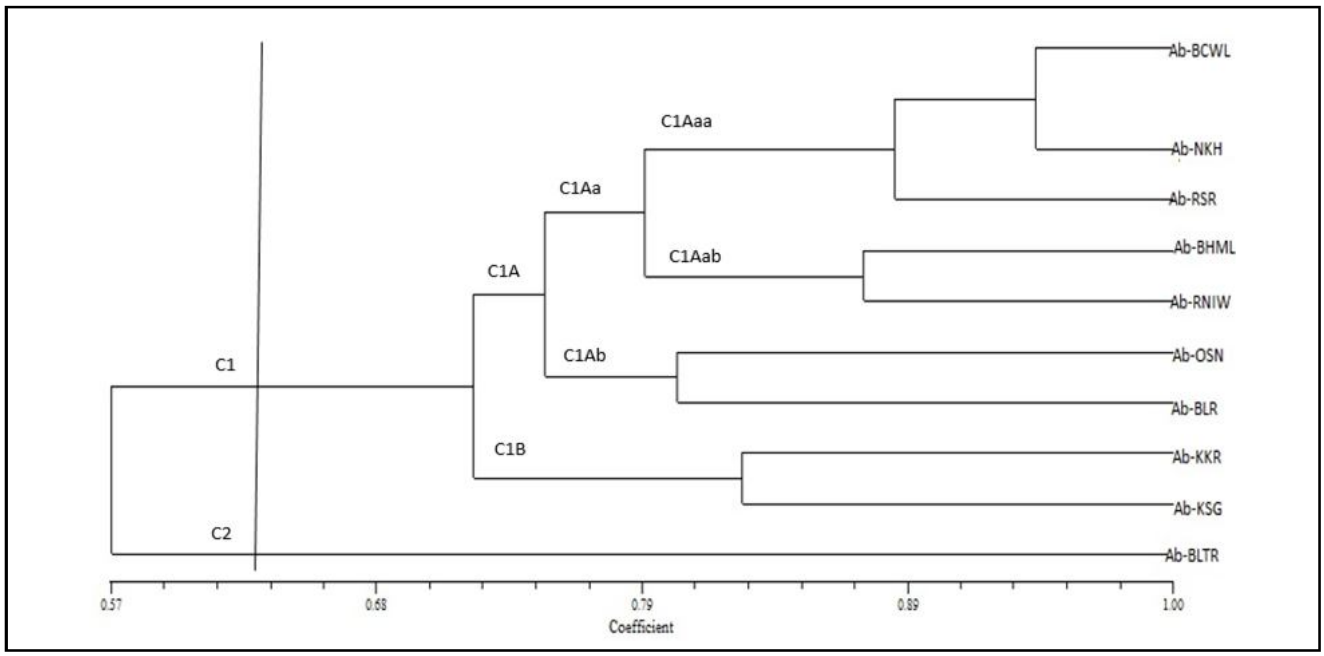

Figure 6: UPGMA dendrogram depicting genetic links among *A. burnsii* isolates based on Jaccard's similarity coefficients and RAPD banding patterns.

4. Discussion

The analysis of genetic similarity matrices using Jaccard's similarity coefficient offers profound insights into both the genetic diversity and geographic distribution patterns of *A. burnsii* isolates across diverse locations within Rajasthan. The discernible high genetic diversity amongst these isolates underscores substantial genetic variation inherent to the species.

Upon a closer examination of the similarity coefficients, intriguing patterns emerge. For instance, the Ab-BCWL isolate displays a higher similarity coefficient with Ab-NKH and Ab-RSR, despite the considerable geographic separation between Beehwal and Ramsar. This discovery posits that genetic relatedness is not solely shaped by spatial proximity, implying the influence of other factors, such as shared evolutionary history or possible gene flow, in governing the genetic similarity observed among these isolates (Christmas *et al.,* 2021). Predominant theories propose that fluctuations in temperature and relative air humidity act as stimuli for the release of spores from plant substrates (Trimmer *et al.,* 1998).

In contrast, isolates hailing from proximate regions like Ramsar and Balotara exhibit lower similarity coefficients. This finding, while unexpected, suggests the presence of discrete genetic lineages or evolutionary pressures molding the genetic constitution of *A. burnsii* isolates within these specific locales.

Remarkably, no distinct clustering of isolates based on geographical origin is evident either in the dendrogram or the similarity matrix. This observation implies the widespread dispersion of *A. burnsii* isolates, negating confinement to particular geographic regions. Such dispersion could be ascribed to the fungus's primary dispersal mode, aerial spores, facilitating long-range migration and colonization opportunities.

The broad genetic diversity and the absence of geographical clustering among *A. burnsii* isolates underscore the intricate nature of the species. The coexistence of diverse genetic lineages and dispersion patterns underscores the potential for adaptation to diverse environmental contexts and the presence of multiple sources of inoculum within the region. Similar findings elucidating genetic diversity in *Alternaria* spp. infecting cumin and other crops and inflicting damage have been reported (Singh *et al.,* 2016; Sharma and Tewari, 1998; and Jabborova *et al.,* 2020).

The principal dispersal mechanism of *Alternaria* species involves the liberation of conidia (asexual spores) into the atmosphere (Woudenberg *et al.,* 2015). Furthermore, it is plausible that the local environment substantially contributes to the observed genetic diversity within populations of aerial *Alternaria*. Particularly, regions characterized by pronounced humidity and temperature variations, coupled with intensive agricultural practices, may foster a milieu conducive to generating diversity that counters selective environmental pressures. This surge in diversity can be attributed to a limited array of evolutionary processes, notably encompassing mutation, recombination or migration (Nielsen and Slatkin, 2013).

In the context of cumin cultivation and blight management, these findings hold immense promise. The recognition of diverse genetic lineages within *A. burnsii* populations necessitates tailored management strategies that account for potential variations in virulence, disease severity and resistance. The absence of geographical clustering highlights the widespread dispersal potential of the pathogen, reinforcing the importance of holistic disease management practices across diverse cumin-growing regions.

Furthermore, the observed genetic diversity also signifies the adaptability of *A. burnsii* to different environmental conditions. This adaptability may influence disease resistance mechanisms and the effectiveness of management strategies. Understanding the genetic landscape of the pathogen can guide the development of targeted interventions, such as resistant cultivars or precision fungicide application, to combat cumin blight effectively.

In conclusion, this study's implications extend beyond elucidating genetic diversity patterns within *A. burnsii* isolates. The findings lay the foundation for improved cumin cultivation practices and proactive blight management, offering the potential to enhance disease resistance strategies and ensure the sustainability of cumin production in the face of evolving pathogenic challenges.

5. Conclusion

The analysis of *A. burnsii* isolates from different locations in Rajasthan revealed a high level of genetic diversity within the isolates. The genetic similarity coefficients indicated that genetic relatedness is not solely determined by geographic proximity, suggesting the influence of other factors such as shared evolutionary history or gene flow. The presence of diverse genetic lineages and dispersal patterns indicates the potential for adaptation to various environmental conditions and multiple sources of inoculum. While this study provides valuable insights into the genetic diversity and relationships of *A. burnsii* isolates, the sample size and geographical scope might not fully capture the entirety of the pathogen's genetic landscape. Additionally, the influence of specific environmental factors and host interactions on the observed genetic patterns requires further investigation. Despite these limitations, the study highlights the complex dynamics underlying *A. burnsii* populations and underscores the need for continuous research in this area.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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