Molecular characterization of *Erysiphe polygoni* isolates on blackgram in Pudukottai district, Tamil Nadu

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ABSTRACT

Blackgram (*Vigna mungo* L) is an important pulse crop in India, contributing significantly to dietary protein and nutritional security. Powdery mildew, caused by *Erysiphe polygoni* DC, is a major disease limiting blackgram productivity across diverse agroclimatic regions. This study investigated the pathogenic, morphological and molecular variability among ten naturally occurring *E polygoni* isolates collected from major blackgram growing areas of Tamil Nadu. The isolates showed noticeable variation in conidial size, hyphal growth, cleistothecia, asci and ascospore number, although traits such as ascus width and ascospore length remained relatively stable. ITS-based PCR amplification yielded a consistent ~630 bp fragment in all isolates, confirming species identity. Molecular analysis revealed high sequence similarity with only minor genetic differences among isolates. Overall, morphological variation was not closely associated with pathogenicity and ITS markers proved effective for species identification but limited for detecting intra-species diversity. The findings support improved pathogen characterization and provide useful information for developing durable powdery mildew resistance in blackgram.

Keywords: Blackgram; *Erysiphe polygoni*; Powdery mildew; Morphological variability; ITS region; Molecular characterization

INTRODUCTION

Pulses are a major source of protein in the Indian diet and play a vital role in sustaining agricultural growth due to their resource conserving and environmentally friendly nature. Increasing pulse production can therefore help address challenges related to food availability and nutritional security (Shalendra et al 2013). India, with >35 Mha pulses cultivation area, is the largest pulses producing country in the world. It ranks first in area and production with 37 and 29 per cent respectively (Anon 2023). In fact, pulses, which constitute a kind of legume, are rich in high quality protein, amino and fatty acids, fibre, minerals and vitamins for vegetarians, who prevail among the Indian population (Singh et al 2017). Protein-energy malnutrition as well as micronutrient deficiencies can be addressed by increasing the consumption of pulses which are a rich source of proteins, minerals, iron and fibre (Rampal 2016).

Blackgram (*Vigna mungo*) is a prominent member of the extensive Leguminosae family. It is highly esteemed among all pulses (Sancho 2023). It contains about 22-24 per cent protein, nearly twice that of wheat and three times that of rice. Blackgram yield is constrained by several biotic and abiotic factors, among which powdery mildew, caused by *Erysiphe polygoni* DC is one of the most serious diseases affecting all aerial parts of the plant (Khandappagol and Rangaiah 2019). The disease is widespread across India and is particularly severe during the dry season (Pandey et al 2009). Although synthetic fungicides are commonly used to manage yield losses, their use poses risks to human health and the environment. Therefore, the development of genetically resistant cultivars

remains a cost-effective and sustainable approach to minimizing losses due to powdery mildew (Khandappagol and Rangaiah 2019).

The present study was undertaken to examine pathogenic variation and characterization of naturally occurring isolates of *E polygoni* from geographically diverse regions of blackgram cultivation.

MATERIAL and METHODS

Collection and maintenance of inoculum of *E polygoni* in greenhouse

Blackgram leaves showing typical powdery mildew symptoms were collected from different districts of Tamil Nadu. Conidial suspensions were prepared according to the methods of Vimala (2005). Conidia were harvested from diseased leaves by flooding the leaves with sterile distilled water. The process was repeated three times to obtain sufficient inoculum. The conidial suspension was strained through two layers of cheese cloth and centrifuged twice at 4,000 rpm for 30 min. The conidial concentration was adjusted to 5×10^6 per ml with sterile distilled water (de Souza and Cafe-Filho 2003). Blackgram CO 5 was used for maintaining the powdery mildew isolates in glasshouse. To maintain the sufficient inoculum, 20 day-old healthy blackgram plants in the greenhouse were sprayed with spore suspension, after making slight pin pricks. They were then covered with polythene bags for 24 h to maintain high humidity for disease development. The different isolates were numbered and maintained for further studies.

Pathogenicity of *E polygoni* in blackgram leaves

Conidial suspension of powdery mildew was prepared by scrapping the conidia from the infected leaves of blackgram. The conidial concentration was adjusted to 10^6 conidia per ml using a haemocytometer. This suspension was sprayed on healthy leaves of 30 days old plants. The plants were covered by polythene bags for 24 hours to maintain high humidity for disease development. The development of the disease was observed at periodical intervals after inoculation (Vimala 2005).

Morphology of *E polygoni* isolates by scanning electron microscopy

Scanning electron microscope (SEM): The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high energy beam of electrons in a raster scan pattern. Here, a wide range of magnifications is possible, from about 10 times (about equivalent to that of a powerful hand lens) to more than 500,000 times. All samples must be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. For taking images of samples, 2 mm leaf sample was placed on the carbon conducting tape. Then the tape was mounted on sample stage and the images were taken in 24,000X magnification and 20 KV using FET SEM Model QUANTA 250 (Raja et al 2025).

Isolation of DNA

Powdery mildew isolates maintained in the glasshouse were used for this study. Total DNA was extracted from conidia of each isolate according to the method described by McDermott et al (1994). Conidia were collected from the infected leaves using camel hair brushes and transferred the conidia into Eppendorf tubes containing 500 µl of extraction buffer {50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 0.7 M NaCl; 1% Cetyltrimethylammonium bromide (w/v) and 1% 2-mercaptoethanol} ($10^9 10^8$ spores/ ml) and vortexed for 30 s and incubated at 60°C for 1 h. The mixture was centrifuged at 13,000 rpm for 10 min and the aqueous phase was added with an equal volume of chloroform:isoamyl alcohol (24:1 v/v) and incubated on a shaker (100 rpm) at room temperature for 1 h. The mixture was centrifuged at 13,000 rpm for 10 min and the aqueous phase was transferred to a new Eppendorf tube and reextracted with chloroform:isoamyl alcohol. The aqueous phase was transferred to a new tube and the DNA was precipitated with equal volume of isopropanol and centrifuged at 13,000 rpm for 10 min. The pellet was washed with 70 per cent ethanol, dried and dissolved in 50 µl of Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0).

Sequencing of the internal transcribed spacer (ITS) region of the rDNA

The purified DNA was amplified by polymerase chain reaction (PCR) using universal ITS1 and ITS4 primers (Table 1).

Reactions were performed in 20 µl mixture containing approximately 50 ng of total DNA, 5 mM each dNTPs, 20 pmol of each ITS1 forward primer and ITS2 reverse primer and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt Ltd, Bangalore,

India). The reaction was carried out in Eppendorf ep gradient S Master cycler (Eppendorf, Hamburg, Germany).

Sequencing of the internal transcribed spacer (ITS) region of the rDNA

The purified DNA was amplified by polymerase chain reaction (PCR) using universal ITS1 (5'-TCC GTA GGT GAA CCT GCG G- 3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC- 3') primers. Reactions were performed in 20 µl mixture containing approximately 50 ng of total DNA, 5 mM of each dNTPs, 20 pmol of each ITS1 forward primer and ITS2 reverse primer and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt Ltd, Bangalore, India). The reaction was carried out in Eppendorf ep gradient S Master cycler (Eppendorf, Hamburg, Germany) programmed with initial denaturation at 94°C for 1 min, followed by 30 cycles of 94°C for 1 min, 58°C for 2 min, 72°C for 1 min and a final extension step at 72°C for 5 min (Table 2). Amplicons were analyzed by electrophoresis in 1.5 per cent agarose gels in TAE buffer and visualized by staining with ethidium bromide and recorded with an Alpha Imager 2000 (Alpha Innotech, San Leandro, CA). The sizes of the PCR products were determined by comparison with standard 100 bp or 1 kb molecular marker (Bangalore Genei Pvt Ltd, Bangalore, India).

PCR products of ~ 630 bp were purified from the gel using QIAquick Gel extraction kit (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's protocol. The purified PCR fragments were cloned into the pGEM-T easy vector (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol and the recombinant plasmids were used to transform *Escherichia coli* strain DH5α. Plasmid DNA was isolated from the clones by using Wizard Plus plasmid DNA purification kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's recommendations. The clones were sequenced in both the forward and reverse directions by the use of forward and reverse primers (SP6 and T7). Sequencing was done at 1st Base Pvt Ltd, Singapore. Database search was performed with the BLAST 2.0 programme from the National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA, World Wide Web server.

RESULTS and DISCUSSION

Characterization of *Erysiphe polygoni* isolates in blackgram

Significant variation occurred among isolates for quantitative and qualitative parameters regardless of the degree of pathogenicity. There was variation in conidium length, conidium width, number of cleistothecia, number of hyphae, number of asci, ascus length, number of ascospores and ascospore width. The virulent isolate (ERPL) produced the 13 hyphae, 98 cleistothesia and 4 asci (Table 3). *E polygoni* isolates indicated high variability for conidium length, conidium width, number of cleistothecia, number of hyphae per unit area, number of asci, ascus length,

Table 1. Primer sequences used for amplification of 18S rDNA

Primer type	Primer sequence	Target
Forward	(5'-TCC GTA GGT GAA CCT GCG G- 3')	18S rDNA
Reverse	(5'-TCC TCC GCT TAT TGA TAT GC- 3')	18S rDNA

Table 2. Thermal cycling conditions used for PCR amplification

Step	Temperature (°C)	Time (min)
Denaturation (initial)	94	3
Denaturation	94	1
Annealing	37	2
Extension	72	2
Extension (final)	72	10

Table 3. Characterization of *Erysiphe polygoni* isolates in blackgram

Isolate	Number of hyphae	Conidial length	Number of cleistothecia	Number of asci	Number of ascospores
ERPP	14	20	70	3	2
ERPN	10	18	84	3	3
ERPV	12	22	82	3	2
ERPC	13	24	76	4	3
ERPT	10	15	82	3	3
ERPA	10	14	72	3	3
ERPM	21	15	94	3	2
ERPo	14	14	89	3	2
ERPL	13	12	98	4	2
ERPE	12	24	86	5	2

number of ascospores and ascospore width. Isolates were not variable for ascus width and ascospore length as indicated by low coefficients of variability viz 10 and 8 per cent respectively. Based on the frequency distribution of qualitative traits, the majority of isolates exhibited ovate conidia (56%) and chained conidia (70%). Most isolates produced black, globose cleistothecia (87%) with asci that were predominantly digitate (52%) or ovate (52%) (Table 4). Elliptical-shaped ascospores were observed in 82 per cent of the isolates (Jeffers 1967, Jolliffe 2002).

Molecular characterization of *E polygoni*

PCR products of ~ 630 bp were purified from the gel using QIAquick Gel extraction kit (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's protocol. The purified PCR fragments were cloned into the pGEM-T easy vector (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol and the recombinant plasmids were used to transform *Escherichia coli* strain DH5α. Plasmid DNA was isolated from the clones by using Wizard Plus plasmid DNA purification kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's recommendations. The clones were sequenced in both the forward and reverse directions by the use of forward and reverse primers. Sequencing was done at 1st Base Pvt Ltd, Singapore. Database search was performed with the BLAST 2.0 programme from the National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA, World Wide Web server. An unweighted pair group method with arithmetic mean phenogram, constructed on the basis of quantitative parameters, indicated that isolates were morphologically different but pathogenicity was not controlled by morphological features of the isolates, though morphological features could be used to distinguish between isolates.

The ITS gene region of fungal DNA is critical for both species and intraspecies molecular systematics. The variation between individual rDNA repeats can occasionally be observed amongst the ITS and IGS regions. The amplicon size of the 18s rRNA region-based primers viz ITS 1 and 4, PMITS 1 and 2 and EryF and EryR on *E pisi* consistent with the reported nucleotide size of *E pisi*, from central and northeast India (Baiswar et al 2015). These findings confirmed the identity of all of the isolates investigated. As a result of these findings, the molecular ITS region could not detect any significant variation within the *E pisi* isolates. Instead, it grouped them all into a single line. Another study showed that such molecular techniques are appropriate and feasible in phylogenetically closely related powdery mildew species assemblages for which ITS investigations do not have widespread value (Hsiao et al 2022).

Plate 1 shows the agarose gel electrophoresis profile of PCR-amplified products obtained from the genomic DNA of *E polygoni*. Lane M represents the molecular weight marker, which was used to estimate the size of the amplified fragments. Lanes 1-10 correspond to different *E polygoni* isolates (ERPP, ERPN, ERPV, ERPC, ERPT, ERPA, ERPM, ERPo, ERPL and ERPE).

All isolates produced a clear and distinct band of approximately 630 bp, indicating successful amplification of the target genomic region. The uniform band size across all lanes confirms the specificity of the primers used and suggests that the amplified region

is conserved among the tested isolates of *E polygoni*. The absence of non-specific bands or smearing further reflects good DNA quality and optimized PCR conditions. Overall, the results validate the reliability of PCR for molecular detection and confirmation of *E polygoni* and support its use in further genetic characterization and diversity studies of the pathogen.

Fig 2 presents the genetic relationships among different isolates of *E polygoni* based on molecular analysis. The isolates are grouped into distinct clusters, reflecting varying degrees of genetic similarity. Closely related isolates such as ERPN and ERPA cluster together, indicated a high level of genetic relatedness, while ERPo and ERPV also show close affinity within

Table 4. Morphological paramenters of *Erysiphe polygoni*

Isolate	Type of conidia	Shaper of conidia	Appearance of cleistothecia	Appearance of asci	Shape of asci	Shape of ascospores
ERPP	Chained	ovate	Globose	Falcate	Ovate	ovate
ERPN	Chained	ovate	Ovate digitate	Digitate	Sub-globose	Eliptical
ERPV	Single	Elipsoidal	Black globose	Digitate	Ovate	Eliptical
ERPC	Single	Ovate	Black globose	Digitate	Globose	Eliptical
ERPT	Chained	ovate	Black globose	Digitate	Globose	Eliptical
ERPA	Chained	Elipsoidal	Black globose	Digitate	Ovate	Eliptical
ERPM	Chained	Ovate	Black globose	Digitate	Sub-globose	Eliptical
ERPo	Chained	ovate	Black globose	Digitate	Ovate	Eliptical
ERPL	Chained	Elipsoidal	Black globose	Digitate	Sub-globose	Eliptical
ERPE	Chained	Ovate	Black globose	Digitate	Ovate	Eliptical

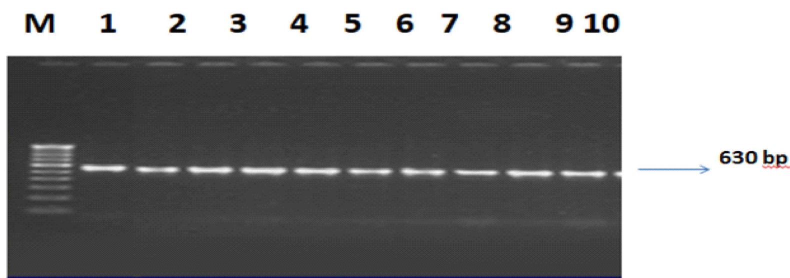

Plate 1. Agarose gel electrophoresis of PCR amplified products from genomic DNA of *Erysiphe polygoni*

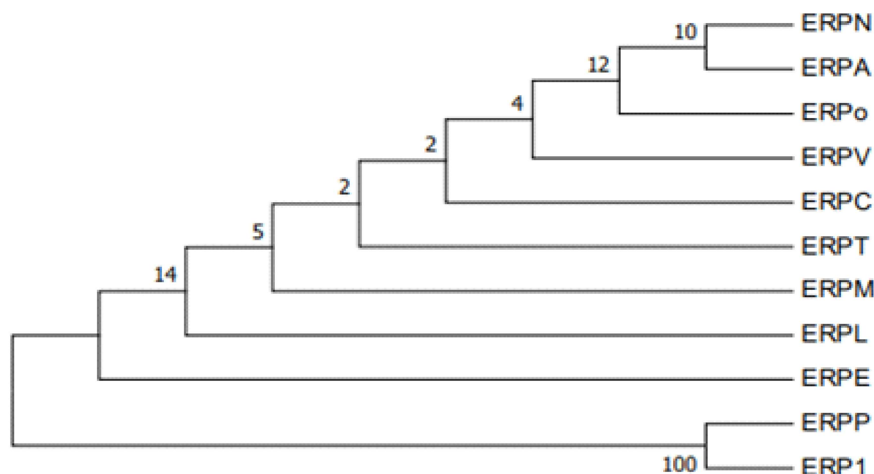

Fig 2. Dendrogram indicating the relationship among the isolates of *Erysiphe polygoni*

the same sub-group. Isolates ERPC, ERPT and ERPM form separate branches, suggested moderate genetic divergence from the above cluster. ERPL and ERPE appeared more distantly related, indicating greater genetic variability among these isolates. Notably, ERPP and ERP1 formed a distinct cluster with a high bootstrap value (100), signifying strong genetic similarity between these two isolates and clear separation from the remaining groups.

Overall, the dendrogram reveals considerable genetic diversity among *E polygoni* isolates. This variability may be associated with differences in geographical origin or host adaptation and has important implications for disease management strategies, particularly for the development of durable powdery mildew resistant blackgram cultivars.

Multiple sequence alignment-based genetic distance among *E polygoni* isolates infecting blackgram is presented in Table 5. Very low or zero distance values among several isolate pairs (eg ERPN, ERPV, ERPC, ERPT and ERPM) indicate a high level of sequence similarity, suggesting that these isolates were genetically very closely related or nearly identical. Slightly higher but still low divergence values were observed for isolates such as ERPA and ERPo when compared with the above group, reflecting minor sequence variations. In contrast, ERPP and ERP1 showed comparatively higher divergence from most other isolates, indicating that they were genetically distinct.

Notably, ERP1 exhibited zero distance with ERPP, confirming their close genetic relationship, which was also consistent with the clustering pattern observed in the dendrogram. Overall, the data demonstrate limited but measurable genetic variability among *E polygoni* isolates, supporting the presence of both closely related and distinct genotypes within the pathogen population.

Fig 3 depicts the similarity matrix derived from multiple sequence alignment of *E polygoni* isolates infecting blackgram. The alignment showed a high degree of sequence conservation across most nucleotide positions, as reflected by the extensive consensus regions. This indicates strong genetic similarity among the isolates, suggesting that a conserved genomic region was targeted in the analysis. Minor nucleotide substitutions were observed at specific positions, accounting for the low levels of genetic variation detected among certain isolates.

These variations were limited and scattered, indicating point mutations rather than major insertions or deletions. Such subtle differences explain the small genetic distances and clustering patterns observed in the dendrogram and alignment tables. Overall, the similarity matrix confirmed that the *E polygoni* population infecting blackgram was largely homogeneous, with only minor sequence divergence among isolates. The conserved nature of the pathogen genome supported the reliability of the molecular marker used and provided useful insights for accurate identification and resistance-breeding programmes.

Plate 2 showcases the scanning electron micrographs of *E polygoni* isolates, revealing a dense, superficial network of branched, septate hyphae that form the characteristic epiphytic mycelial mat. The reproductive morphology is defined by the production of distinct ellipsoidal to barrel-shaped conidia which exhibit a slightly granular surface texture under high magnification, a key diagnostic feature for the species.

Table 5. Multiple alignment of *Erysiphe polygoni* on blackgram isolates

Isolate	ERPP	ERPN	ERPV	ERPC	ERPT	ERPA	ERPM	ERPo	ERPL	ERP1
ERPN	0.357									
ERPV	0.357	0.000								
ERPC	0.357	0.000	0.000							
ERPT	0.357	0.000	0.000	0.000						
ERPA	0.389	0.013	0.013	0.013	0.013					
ERPM	0.357	0.000	0.000	0.000	0.000	0.013				
ERPo	0.371	0.007	0.007	0.007	0.007	0.020	0.007			
ERPL	0.357	0.000	0.000	0.000	0.000	0.013	0.000	0.007		
ERPE	0.357	0.000	0.000	0.000	0.000	0.013	0.000	0.007	0.000	
ERP1	0.000	0.357	0.357	0.357	0.357	0.389	0.357	0.371	0.357	0.357

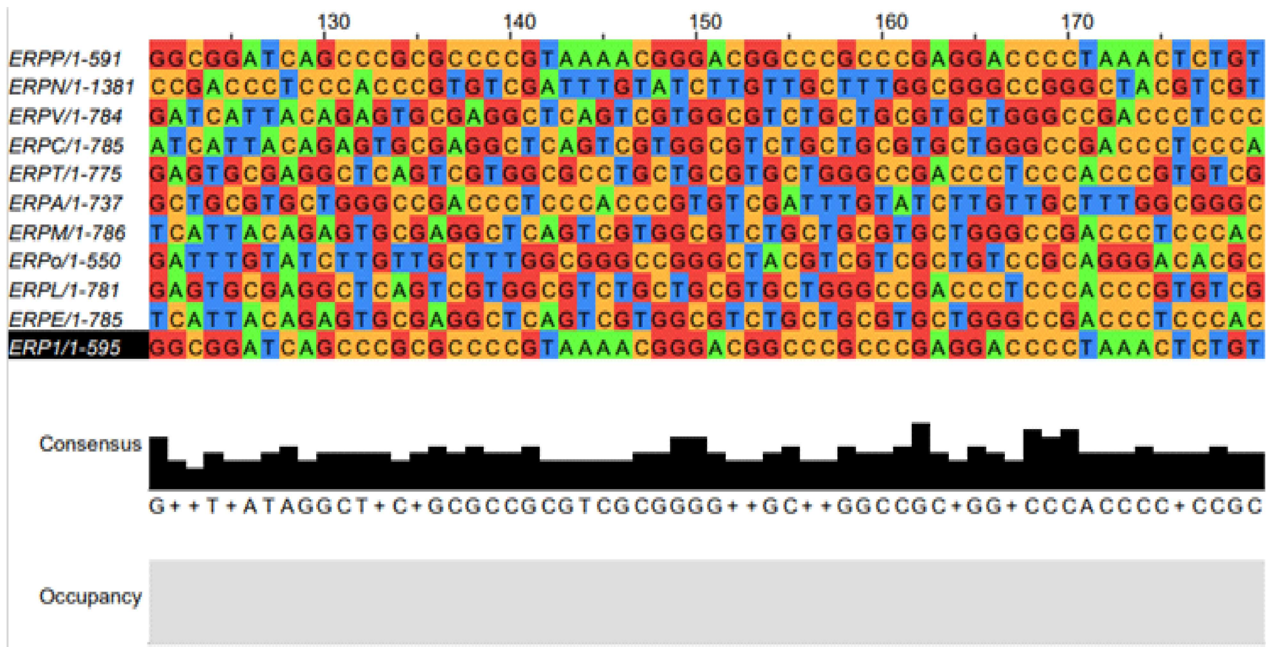

Fig 3. Similarity matrix of *Erysiphe polygoni* in blackgram

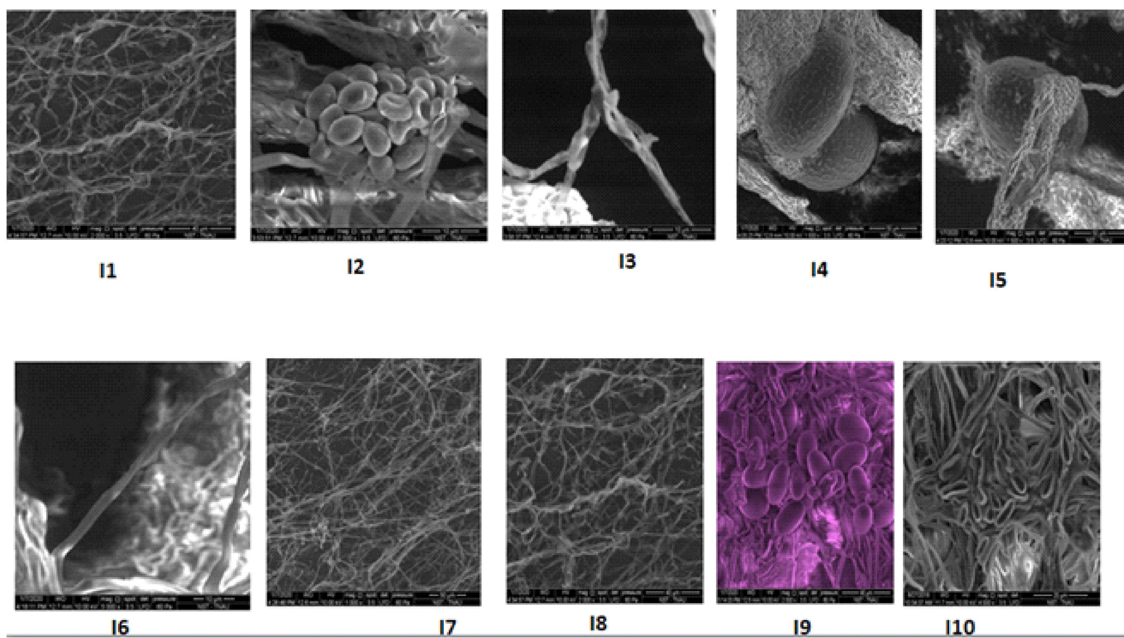

Plate 2. Scanning electron micrographs showing the morphology of *Erysiphe polygoni* isolates

CONCLUSION

The present study revealed significant morphological variability among *Erysiphe polygoni* isolates infecting blackgram, particularly in conidial traits, cleistothecia formation and ascus characteristics, indicating the presence of phenotypic diversity within the pathogen population. However, this morphological variation was not consistently associated with pathogenicity levels. Molecular analysis based on the ITS region confirmed the identity of all isolates as *E polygoni* and showed a high degree of sequence conservation, suggesting limited genetic diversity at the intra-species level. The clustering pattern and genetic distance analysis further indicated that ITS markers are reliable for species level identification but have limited discriminatory power for differentiating closely related isolates. Overall, the combined use of morphological, pathogenic and molecular approaches provided a comprehensive understanding of *E polygoni*

variability. These findings are valuable for disease diagnostics and emphasize the need for incorporating diverse pathogen populations while developing durable powdery mildew resistant blackgram cultivars.

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REFERENCES

Anonymous 2023. Annual report 2022-23. Directorate of Pulses Development, Department of Agriculture and Farmers' Welfare, Ministry of Agriculture and Farmers' Welfare, Government of India, Bhopal, Madhya Pradesh, India.

Baiswar P, Ngachan SV, Verma VK, Kumar R, Jha, AK and Chandra S 2015. Molecular evidence of *Erysiphe pisi* on pea and *E trifoliorum* on white clover in northeast India. Australasian Plant Disease Notes **10:** 12; doi: 10.1007/s13314-015-0165-2.

de Souza VL and Cafe-Filho AC 2003. Resistance to *Leveillula taurica* in the genus *Capsicum.* Plant Pathology **52:** 613-619; doi: 10.1046/j.1365-3059.2003.00920.x.

Hsiao H-Y, Ariyawansa HA, Hsu C-C, Wang C-J and Shen Y-M 2022. New records of powdery mildews from Taiwan: *Erysiphe ipomoeae* comb nov, *E aff betae* on buckwheat and *E neolycopersici* comb nov on *Cardiospermum halicacabum*. Diversity **14(3):** 204; doi: 10.3390/d14030204.

Jeffers JNR 1967. Two case studies in the application of principal component analysis. Journal of the Royal Statistical Society Series C: Applied Statistics **16(3):** 225-236; doi: 10.2307/2985919.

Jolliffe IT 2002. Principal component analysis. 2nd Edn, Series in Statistics. Springer, New York, 488p.

Khandappagol M and Rangaiah S 2019. Evaluation of blackgram (*Vigna mungo* L Hepper) genotypes for resistance against powdery mildew (*Erysiphe polygoni* DC) under natural and artificially inoculated conditions. International Journal of Current Microbiology and Applied Sciences **Special Issue 9:** 171-182.

McDermott JJ, Schukken YH and Shoukri MM 1994. Study design and analytic methods for data collected from clusters of animals, Preventive Veterinary Medicine **18(3):** 175-191; doi: 10.1016/0167-5877(94)90074-4.

Pandey S, Sharma M, Kumari S, Gaur PM, Chen W, Kaur L, MacLeod W, Basandrai A, Basandrai D, Bakr A, Sandhu JS, Tripathi HS and Gowda CLL 2009. Integrated foliar diseases management of legumes. In: Proceedings of the International Conference on Grain Legumes: Quality Improvement, Value Addition and Trade, 14-16 February 2009, Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India, pp 143-161.

Raja K, Chinnasamy GP, Alex VA, Parameswari K, Vijayageetha V and Pradeep D 2025. ZnO nanoparticles seed invigouration for the maintenance of seed vigour and viability in black gram. Legume Research **48(12):** 2014-2023; doi: 10.18805/LR-5267.

Rampal P 2016. Pulse consumption in India. Foundation for Agrarian Studies, 16 December 2016.

Sancho ANR 2023. Unveiling the potential of blackgram: a nutrient-rich pulse for sustainable agriculture. Biotica Research Today **5(9):** 683-685.

Shalendra, Gummagolmath KC, Sharma P and Patil SM 2013. Role of pulses in the food and nutritional security in India. Journal of Food Legumes **26(3-4):** 124-129; doi: 10.59797/jfl.v26i3&4.879

Singh B, Singh JP, Shevkani K, Singh N and Kaur A 2017. Bioactive constituents in pulses and their health benefits. Journal of Food Science and Technology **54:** 858-870; doi: 10.1007/s13197-016-2391-9.

Vimala R 2005. Studies on powdery mildew of bhendi caused by *Erysiphe cichoracearum* (DC). PhD Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, pp 120; URL: https://krishikosh.egranth.ac.in/handle/1/5810188924.

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