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Unveiling Morphological and Molecular Characteristics of Powdery Mildew on Cucumbers (*Cucumis sativus* L.) in Southern Karnataka, India

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Abstract

Powdery mildew is a significant fungal disease affecting cucumber (*Cucumis sativus* L.), causing substantial yield losses in cucurbit crops worldwide. This study focuses on the morphological and molecular characterization of the powdery mildew pathogens infecting cucumber. Roving field surveys conducted in different locations of Bengaluru Rural district revealed varying disease severities across farmer fields. The highest Percentage Disease Index (PDI) for powdery mildew was recorded in Bagalur and Thimmasandra. Based on the morphological characteristics of the conidial and conidiophore structures, the fungus was identified as *Podosphaera xanthii*. This identification was further confirmed through molecular analysis using universal ITS primers, species-specific primers, DNA sequencing and phylogenetic analysis to ensure accurate pathogen classification. The findings provide critical insights into pathogen diversity and pave the way for developing improved management strategies tailored to resistant cucumber genotypes.

Keywords : Powdery Mildew, Cucumber, Pathogen identification, Morphology, Molecular, Phylogeny

Cucumber, a vital member of the *Cucurbitaceae* family, is a widely cultivated crop grown in both protected environments and open fields across the globe, primarily for its culinary and nutritional value (Harshitha & Shyamalamma, 2021 and Swathi, 2022). Cucumber is rich in essential vitamins B and C is an important dietary component in many cultures. Globally, it is cultivated on approximately 2.23 million hectares, yielding about 87.8 million tons annually (Faostat, 2021). In India, cucumber is grown on 116 hectares, producing a total yield of 1,608 metric tons. Karnataka, a leading state in cucumber production, plays a significant role in its cultivation, particularly in regions such as Mysore, Doddaballapur, Hoskote, Anekal and the northern parts of the state, where it is predominantly grown during the summer season.

Powdery mildew is a serious fungal disease affecting cucurbits, including cucumber, resulting in considerable yield losses and diminished fruit quality. It is caused by obligate biotrophic fungi, primarily *Podosphaera xanthii* and *Golovinomyces cichoracearum* (Perez-Garcia *et al.,* 2009 and Dharshini *et al.,* 2015). The disease is first observed as white, powdery mycelial growth on the surface of leaves, stems and petioles (Fig. 1). As the disease progresses under favorable environmental conditions, secondary symptoms such as chlorosis, premature leaf senescence and defoliation may develop, significantly affecting plant health and productivity.

Although powdery mildew is rarely fatal to the host, its impact on cucumber plants can be severe, reducing yields by up to 40 per cent. This loss is attributed to the fungus's ability to absorb nutrients from the host tissues, hinder normal plant growth and reduce photosynthetic efficiency. The resulting weakened plants produce smaller, lower-quality fruits, which

negatively affect marketability and economic returns for growers. The disease thrives in warm and dry climates but requires high humidity for spore germination and successful pathogen spread. The unique environmental adaptability of the pathogen allows it to persist across diverse agroecological zones, making it highly prevalent in tropical, subtropical and temperate regions worldwide (Nayak *et al.,* 2023).

Powdery mildew is a pervasive fungal disease that affects a wide range of crops (Agrios, 2005 and Glawe, 2008), including cucumbers (*Cucumis sativus* L.). The disease is caused by several species within the *Erysiphaceae* family (Ascomycota; Erysiphales) (Takamatsu, 2018). In cucurbits, powdery mildew is predominantly caused by the obligate biotrophic fungi *Podosphaera xanthii* (syn. *P. fusca*) (Castagne) U. Braun and N. Shishkoff and / or *Golovinomyces cichoracearum* (DC.) V.P. Heluta. *P. xanthii* thrives during the warmer months (Rur *et al.,* 2017), whereas *G. cichoracearum* is more prevalent during the cooler spring and early summer months (Aguiar *et al.,* 2012). Distinguishing between these species requires examining the morphology of the conidia and identifying the presence of fibrosin bodies within the conidia.

The classification of powdery mildew fungi has undergone significant revisions with the advent of DNA-based studies, emphasizing the need for detailed morpho-molecular identification for effective disease management (Heffer *et al.,* 2006). This study investigates the morpho-molecular characteristics of powdery mildew affecting cucumbers, focusing on southern Karnataka, where cucumber is an important crop. The primary objective of the current study aims to investigate the occurrence, morphological and molecular characterisation of powdery mildew pathogen infecting cucumber in Southern Karnataka.

Material and Methods

Survey and Disease Assessment

Roving surveys were conducted during the *rabi* and *kharif* seasons 2022-2023 across major cucumber-growing regions in the Bengaluru Rural district of Karnataka. The surveyed areas included Bagalur, Thimmasandra, GKVK campus (Bengaluru), Hesseraghatta and Devanahalli, areas known for significant cucumber cultivation. Cucumber plants exhibiting characteristic symptoms of powdery mildew, such as white powdery fungal growth on leaves, stems, and petioles, were systematically collected from these locations for detailed study. The severity of the disease was assessed by calculating the Percentage Disease Index (PDI) based on the 0–9 scale proposed by Mayee and Datar (1986). Disease severity levels were categorized into five classes: very low (0–10%), low (11–25%), medium (26–50%), high (51–75%) and very high (76–100%), providing a clear framework for understanding the extent of disease prevalence and impact in each region.

Morphological Analysis

From the infected leaf samples of cucumber, the white powdery colonies/patches were collected using a sterile camel brush and dipped in sterile distilled water to microscopically examine the morphology of the conidia, conidiophores, fibrosin bodies and other relevant fungal structures using LEICA DH750 microscopic image software. Measurements were taken for conidial size, shape, conidiophore, pattern of hyphal growth and the presence or absence of fibrosin bodies (Nayak, *et al*., 2023).

DNA Extraction and Sequencing

Total DNA was extracted from 13 fungal samples using 5 per cent Chelex-100 method (Liu *et at*., 2015) and specific molecular markers (e.g., ITS region, S1/S2, L1/L2 and G1/G2 specific to *Podosphaera xanthi*, *Leveilulla taurica* and *Golovinomyces cichoracearum*, respectively) (Table 1) (Chen *et al*., 2008). The PCR products were purified and Sanger sequenced to determine the genetic makeup of the pathogens. The PCR reaction mixture (20 µL) included TAKARA master mixture (10.0 µL), forward primer (1.0 µL), reverse primer (1.0 µL), PCR water (6.0 µL) and template DNA (2.0 µL). The amplification was carried out with initial denaturation of 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 2 minutes and the final extension step at 72°C for 7 minutes.

Table 1

List of primers

Primer code	Specific molecular markers	Organism
ITS5/4	ITS-5: 5'-GGAAGTAAAAGTCGTAACAAGG-3'ITS-4: 5'-TCCTCCGCTTATTGATATGC-3'	Universal for fungi
S1/S2	5'- GGA TCA TTA CTG AGC GCG AGG CCC CG -3'/5'-CGC CGC CCT GGC GCG AGA TAC A -3'	*Podosphaera xanthi*
G1/G2	5'- TCC GTA GGT GAA CCT GCG GAA GGA T -3'/5'-CAA CAC CAA ACC ACA CAC ACG GCG -3'	*Golovinomyces cichoracearum*
L1/L2	5'- CCC TCC CAC CCG TGT CGA CTC GTC TC -3'/5'-CTG CGT TTA AGA GCC GCC GCG CCG AA -3'	*Leveilulla taurica*

Following PCR amplification, the resulting products were analyzed by agarose gel electrophoresis (1% w/v) to confirm their presence and size, which was expected to be approximately 600 base pairs for the ITS region. The gels were stained with ethidium bromide (10 µg/mL) and visualized using a gel documentation system to verify the successful amplification of the targeted DNA regions. Subsequently, the amplified DNA products were sequenced in both forward and reverse directions at Medauxin, Bangalore, India.

The full-length sequence of ITS region of *P. xanthi* isolate was queried in NCBI database using Blast tool to find similar sequences available in the database. The sequences of different *Podosphaera* infecting various crops were retrieved from the NCBI database and aligned using BioEdit (Hall, 1999) and ClustalW (Thompson *et al.*, 1994) programmes. To know the evolutionary relationship of the test sequences a phylogenic analysis was performed by comparison with the sequences retrieved from the NCBI GenBank database using Neighbor-joining method MEGA X software with 1000 bootstrapped replications (Tumara *et al*., 2021).

Results and Discussion

Symptoms of Powdery Mildew on Cucumber

Symptoms of powdery mildew on cucumber are characterized by the development of white, powdery mycelial mats on both the upper (adaxial) and lower (abaxial) surfaces of leaves. These powdery growths may also appear on stems and fruits, significantly affecting the plant's photosynthetic efficiency and overall vigor (Fig. 1). Disease severity was assessed using the Percentage Disease Index (PDI) for powdery mildew isolates (C1–C13) collected from various locations.

The results revealed substantial variation in disease incidence across the surveyed regions (Fig. 2). Among the surveyed locations, Bagalur (97.78%) and Thimmasandra (95.56%) recorded the highest PDI, indicating severe disease incidence and widespread infection. Similarly, the GKVK campus, Bengaluru and Mattabarlu exhibited high PDI around 88.89 per cent, further highlighting the prevalence of the disease in these areas (Table 2). Conversely, locations such as Nayanadahalli, Upparpete and Hesseraghtta displayed moderate PDI, suggesting a relatively lower disease burden. Areas like Nayanahalli, Chikka Tumkur, Devanahalli and Guttahalli exhibited the lowest PDI ranging from 71.11 to 73.33 per cent, indicating milder disease impact in these regions (Table 2). The observed variability in powdery mildew severity across locations underscores the influence of environmental conditions, host plant susceptibility and pathogen virulence on disease development. Regions with higher PDI may have experienced more favorable conditions for the pathogen, such as higher humidity, temperature variations or dense planting practices, which facilitate disease proliferation.

Morphological identification : The morphological analysis revealed the presence of elliptical conidia,

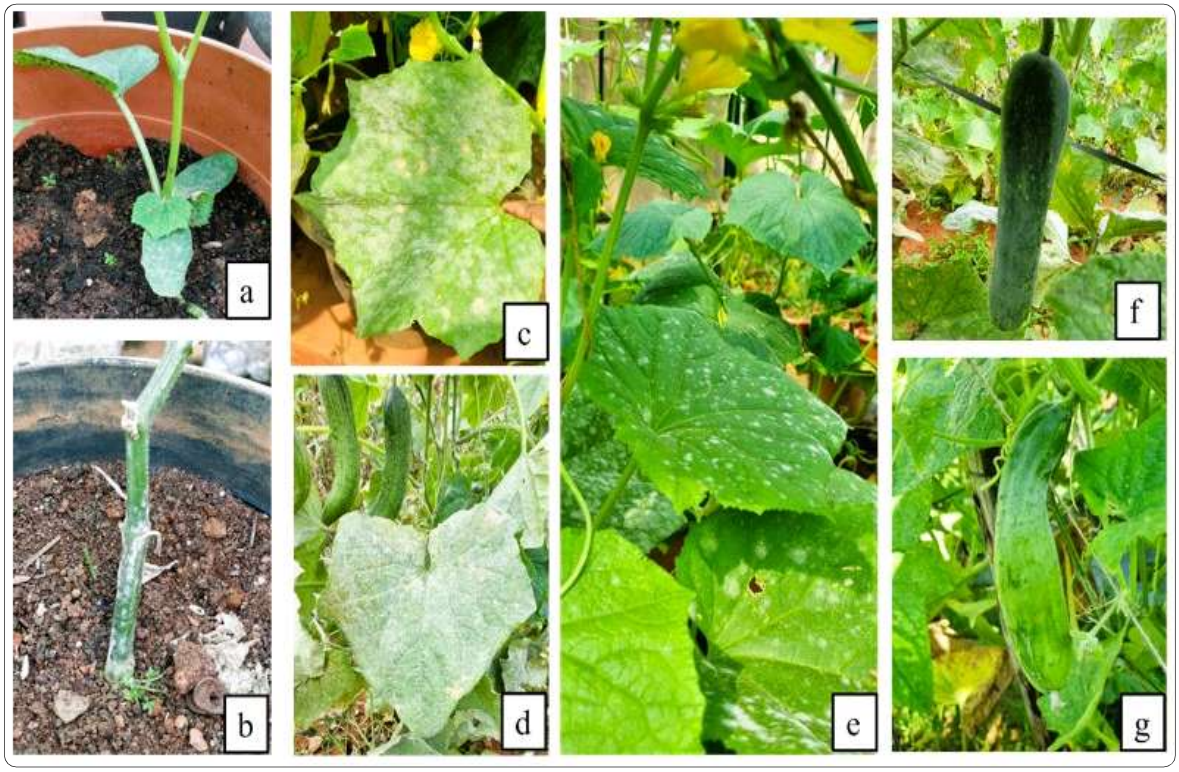

Fig. 1 : Symptoms of powdery mildew on cucumber a) Cotyledons; b) Stem; c, d & e) Leaves and f) Fruits

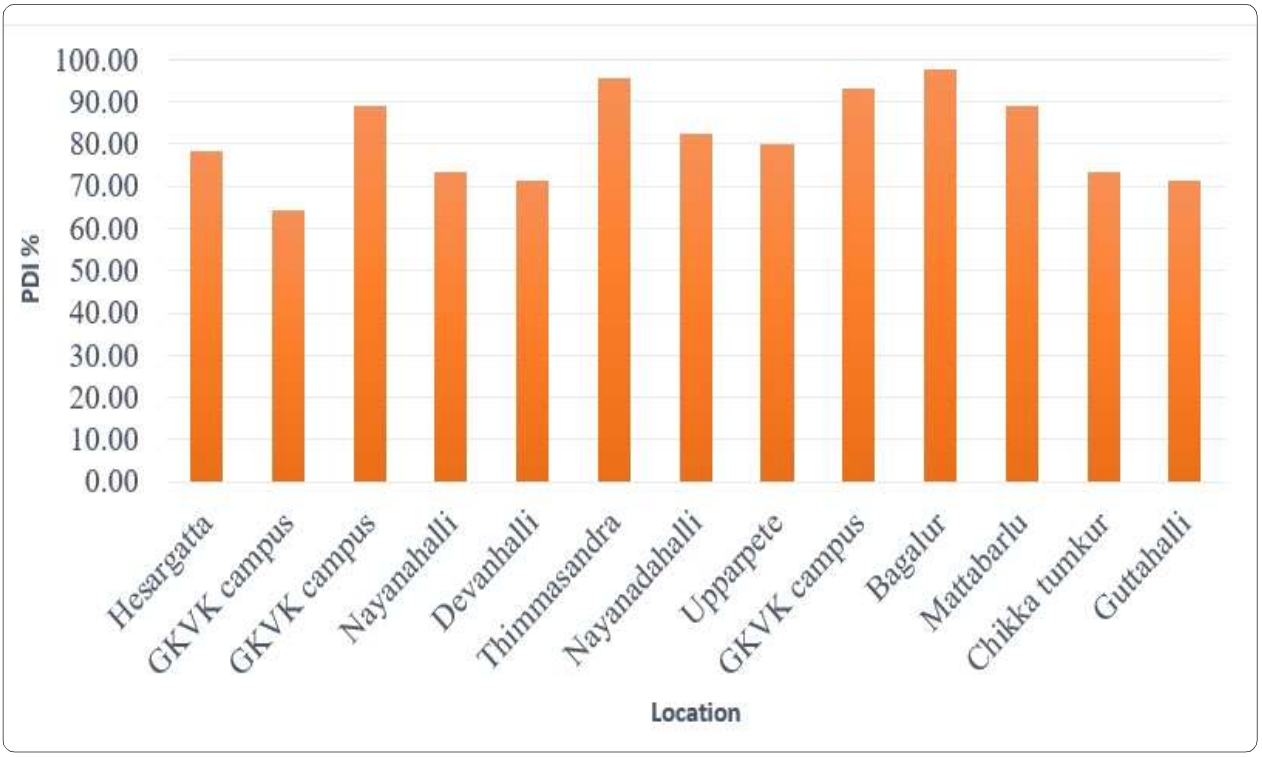

Fig. 2 : The bar graph represents the PDI of powdery mildew on cucumber collected from different locations

TABLE 2

Cucumber isolates with location and severity of powdery mildew disease

Isolates	Location	PDI
C1	Hesargatta	78.43
C2	GKVK campus	64.44
C3	GKVK campus	88.89
C4	Nayanahalli	73.33
C5	Devanhalli	71.11
C6	Thimmasandra	95.56
C7	Nayanadahalli	82.22
C8	Upparpete	80.00
C9	GKVK campus	93.33
C10	Bagalur	97.78
C11	Mattabarlu	88.89
C12	Chikkatumkur	73.33
C13	Guttahalli	71.11

unbranched conidiophores and fibrosin bodies, characteristic of *Podosphaera xanthii*. The conidia were predominantly ellipsoid to ovoid with dimensions ranging from 25-35 µm in length and 15-20 µm in width matching with Nayak *et al*. (2023). The conidiophores were erect, septate and measured between 100-200 µm in height (Fig. 3). Hyphal, conidiophore and conidial characteristics, along with conidial germination (fibroidium type with lateral to sub-terminal germ tube) and presence of fibrosin bodies matched the morphological description of *P. xanthii* (Braun and Cook, 2012). This fungus has been previously identified as the causal agent of powdery mildew in various cucurbit crops (Morishita *et al*., 2003; Gogoi *et al*., 2013 and Moradi *et al*., 2017). Gregorio *et al.* (2022) also reported *P. xanthii* as the predominant species causing powdery mildew in cucurbits.

Molecular Identification: The identity of fungal pathogens is confirmed through PCR amplification by targeting specific DNA sequences using appropriate primers. Initially, universal ITS primers, which amplify the Internal Transcribed Spacer (ITS) region, are used for broad fungal identification due to its conserved and species-diagnostic properties. For species-specific confirmation, primers such as S1/S2 (for *Podosphaera xanthii*), G1/G2 (for *Golovinomyces cichoracearum*) and L1/L2 (for *Leveillula taurica*) are employed. In this study, the S1/S2 primers amplified DNA fragments between 500-600 base pairs (bp), confirming *P. xanthii* as the causal organism of powdery mildew in cucumber isolates. In contrast, the G1/G2 and L1/L2 primers showed no amplification in cucumber, as *G. cichoracearum* and *L. taurica* are

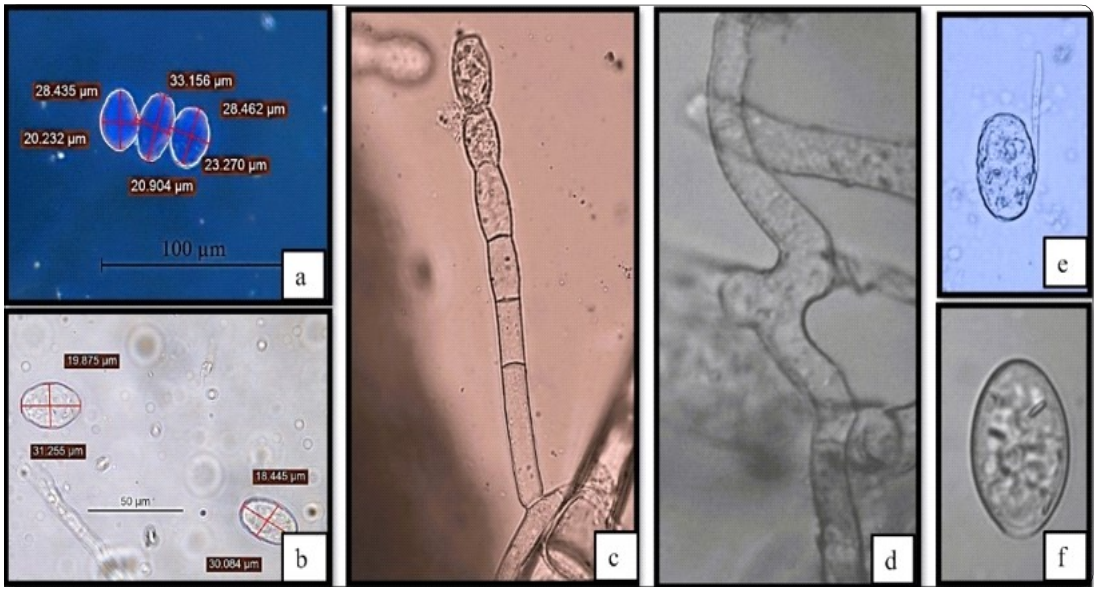

Fig. 3 : a,b) Conidia; c) Conidiophore; d) appressorium; e) germinating conidia; f) fibrosin bodies of *P. xanthi* infecting cucumber

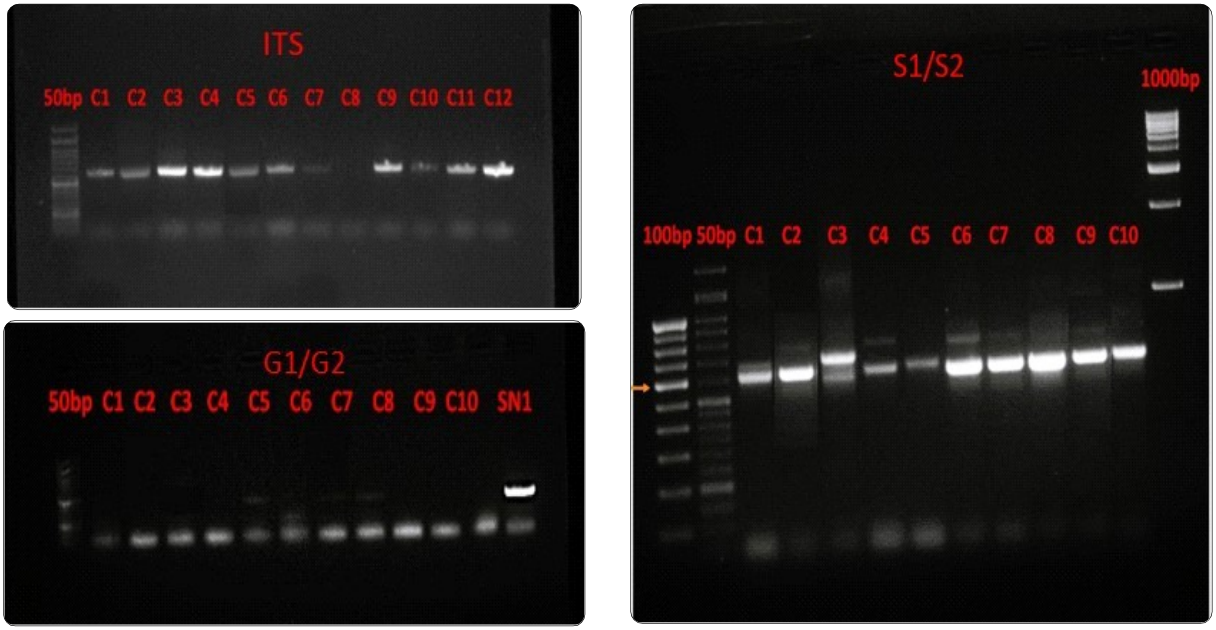

Fig. 4 : Gel picture showing amplified products of cucumber powdery mildew fungal isolates using ITS, S1/S2 and G1/G2 primers

not known to infect this crop. A sunflower positive control (SN1) used with G1/G2 produced a clear b and validating the functionality of these primers. Gel electrophoresis was utilized to visualize PCR products and the presence of bands at expected sizes confirmed the pathogen identity (Fig. 4).

Further verification was achieved through phylogenetic analysis, which grouped the pathogen into distinct clades alongside related species, reflecting evolutionary divergence and affirming the specificity of the primers. Ten sequences from isolate C1-C10 were individually aligned with reference sequences of other *Podosphaera* species, while sequences from *Golovinomyces* were used as an outgroup for comparative analysis. A phylogenetic tree was constructed using the maximum likelihood method in MEGA X software. The resulting phylogenetic tree, based on ITS sequences, demonstrated that the isolates shared the highest percentage identity with *Podosphaera xanthii*, clustering closely with related species in a distinct clade (Fig. 5).

The internal transcribed spacer (ITS) region is widely recognized as a reliable DNA barcode for fungal identification and phylogenetic studies, particularly in research focusing on the *Erysiphales* order (Cunnington *et al.,* 2003; Kovịcs *et al.,* 2011 and Wang *et al.,* 2013). Hirata and Takamatsu (1996) successfully sequenced the ITS region of rDNA from four powdery mildew genera and demonstrated that this region, along with other non-coding regions such as the intergenic spacer (IGS), exhibits significant variability. This variability makes these regions highly suitable for resolving phylogenetic relationships among closely related species. Numerous studies have since confirmed the utility of ITS sequences in identifying powdery mildew pathogens (Moparthi *et al.,* 2018; Smith *et al.,* 2021 and Qasim *et al.,* 2022).

In this study, the integration of morphological and molecular analyses provided a robust framework for the precise identification of powdery mildew pathogens infecting cucumber. Morphological observations, such as the structure of conidia and conidiophores were complemented by molecular techniques, including ITS sequencing and phylogenetic analysis. The results confirmed the dominance of *Podosphaera xanthii* as the primary causal agent of powdery mildew on cucumber in the study region. Furthermore, the molecular data revealed notable genetic diversity within *P. xanthii*, indicating

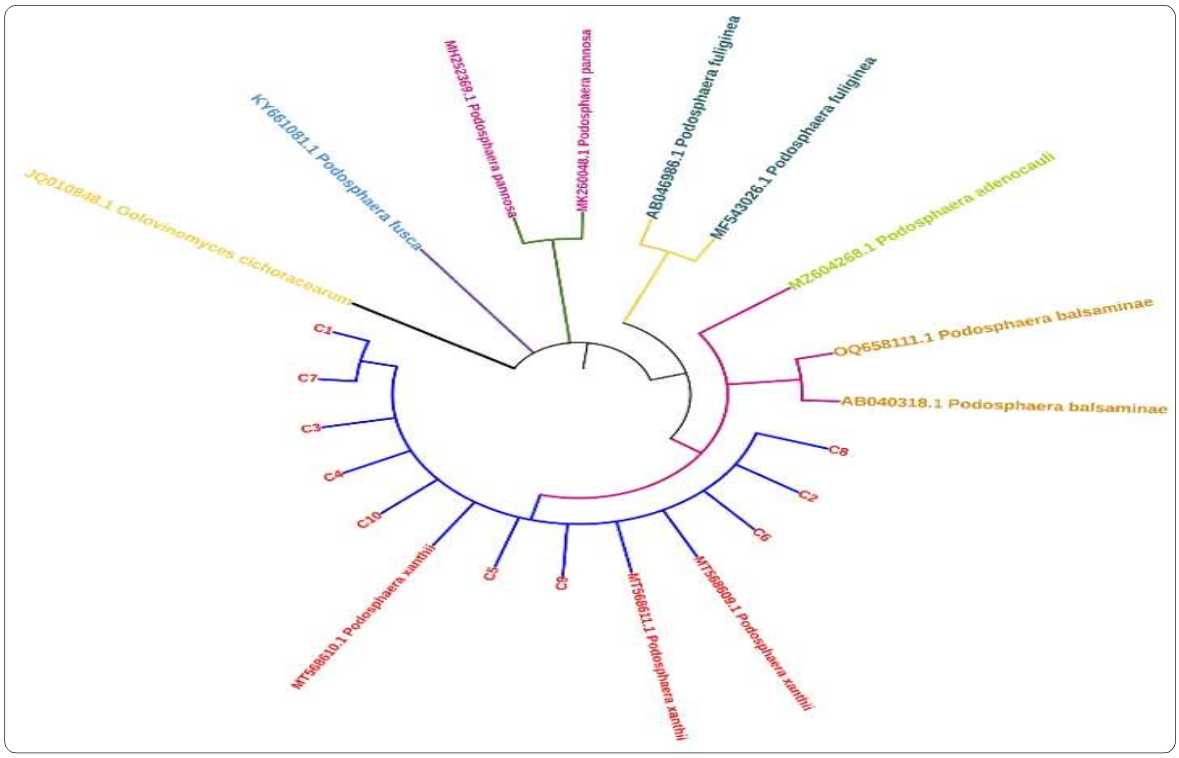

Fig. 5 : Phylogenetic tree constructed from nucleotide sequences of *ITS* region of *Podosphaera* isolate infecting cucumber with sequences of related species retrieved from NCBI GenBank

the presence of multiple strains or closely related variants of this pathogen.

This genetic diversity has significant implications for disease management. Variability among strains may result in differences in virulence, host specificity and resistance to commonly used fungicides. Effective management strategies must account for this diversity to ensure their efficacy and sustainability. For example, incorporating resistant cucumber genotypes, optimizing the use of fungicides and deploying integrated disease management practices are essential for mitigating the impact of powdery mildew.

The findings of this study also contribute to our broader understanding of pathogen diversity and evolution within the *Erysiphales* order. By combining morphological characterization with molecular tools, this research advances the identification and classification of powdery mildew pathogens. Such insights are critical for developing targeted and sustainable disease management strategies that minimize yield losses and enhance the resilience of cucumber cultivation systems against powdery mildew outbreaks.

References

Agrios, G. N., 2005, Plant pathology 5th Edition: Elsevier Academic Press. *Burlington, Ma. USA*, pp. : 79 - 103.

Aguiar, B. D. M., Vida, J. B., Tessmann, D. J., Oliveira, R. R. D., Aguiar, R. L. and Alves, T. C. A, 2012, Fungal species that cause powdery mildew in greenhouse-grown cucumber and melon in Parana State, Brazil. *Acta Sci. Agron.,* **34** (3) : 247 - 252.

Braun, U. and Cook, R. T. A., 2012, Taxonomic manual of the *Erysiphales* (powdery mildews), vol. 11, *CBS Biodiversity Series*. CBS Biodiversity Series No. 11. CBS-KNAW Fungal Biodiversity Centre, The Netherlands.

Chen, R. S., Chu, C., Cheng, C. W., Chen, W. Y. and Tsay, J. G., 2008, Differentiation of two powdery mildews

of sunflower (*Helianthus annuus*) by PCR-mediated method based on ITS sequence. *Eur. J. Plant Pathol*, **121** (3) : 1 - 8.

Cunnington, J. H., Takamatsu, S., Lawrie, A. C. and Pascoe, I. G., 2003, Molecular identification of anamorphic powdery mildews (*Erysiphales*). *Australas. Plant Pathol.*, **32** : 421 - 428.

Dharshini, G. M., Jagadish, K. S., Shadakshari, Y. G. and Karuna, K., 2015, Spatial distribution of mycophagous coccinellid, *Illeiscincta* Fab. (Coleoptera: *Coccinellidae*) and powdery mildew infection in sunflower, *Helianthus annuus* L. *Mysore J. Agric. Sci*., **49** (2) : 321 - 323.

Foastat. Food and Agriculture Organization of the United Nations. Available online: https://www.fao.org/faostat/en/#search/Cucumbers % 20 and % 20 gherkins (accessed on 14 November 2021).

Glawe, D. A., Windom, G. E., Grove, G. G. and Falacy, J. S., 2003, First report of powdery mildew of *Convolvulus arvensis* (field bindweed) caused by *Erysiphe convolvuli* var. *convolvuli* in North America. *Pl. Health Prog*., **4** (1) : 31.

Gogoi, R., Singh, P. K., Kumar, R., Nair, K. K., Alam, I., Srivastava, C., Yadav, S., Gopal, M., Choudhury, S. R. and Goswami, A., 2013, Suitability of Nano-sulphur for biorational management of powdery mildew of okra (*Abelmoschus esculentus* Moench) caused by *Erysiphe cichoracearum*. *J. Pl. Pathol. Microbiol.*, **4** : 4.

Gregorio-Cipriano, R., Gonzalez, D., Felix-Gastelum, R. and Chacon, S., 2022, Morphological and molecular identification of new records and new host plants of powdery mildews (*Erysiphaceae*) from Mexico. *Bot.*, **100** (7) : 533 - 549.

Hall, T. A., 1999, Bioedit: A user-friendly biological sequence alignment editor and analysis programme for windows 95/98/nt. *Nucleic Acids Symposium Series*, **41** (41) : 95 - 98.

Harshitha, C. K. and Shyamalamma, S., 2021, Morphological characterization of local cucumber (*Cucumis sativus* L.) genotypes for fruit quality traits. *Mysore J. Agric. Sci.*, **55** (4) : 130 - 141.

Heffer, V., Johnson, K. B., Powelson, M. L. and Shishkoff, N., 2006, Identification of powdery mildew fungi. *Plant Health Instr*., **12** : 2006 - 2016.

Hirata, T. and Takamatsu, S., 1996. Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. *Mycoscience*, **37** : 283 - 288.

Kovacs, G. M., Jankovics, T. and Kiss, L., 2011, Variation in the nrDNA ITS sequences of some powdery mildew species: do routine molecular identification procedures hide valuable information? *Eur. J. Plant Pathol.*, **131** : 135 - 141.

Liu, L., Wang, C. L., Peng, W. Y., Yang, J., Lan, M. Q., Zhang, B., Li, J. B., Zhu, Y. Y. and Li, C. Y., 2015, Direct DNA extraction method of an obligate parasitic fungus from infected plant tissue. *Genet. Mol. Res.*, **14** (4) : 18546 - 18551.

Mayee, C. D. and Datar, V. V., 1986, *Phytopathopmety. Technical Bulletin-1* (special bulletin-3), Marathwada Agricultural University, Parbhani, Maharashtra, India, pp. : 29.

Moparthi, S., Bradshaw, M. J. and Grove, G. G., 2018, First report of powdery mildew caused by *Golovinomyces spadiceus* on *Helianthus annuus*. *Plant Dis.*, **102** : 1176.

Moradi, N., Rahimian, H., Deshistani, A. and Babaeizad, V., 2017, Comparative study of selected cucumber cultivar resistant to powdery mildew caused by *Sphaerotheca fuliginea*. *Pl. Cell Biotech. Mol. Biol.*, **18** (1&2) : 30 - 38.

Morishita, M., Sugiyama, K., Saito, T. and Sakata, Y., 2003, Powdery mildew resistance in cucumber. *Jpn. Agric. Res. Q.*, **37** : 7 - 14.

Nayak, A. M., John, P. and Dhange, P. R., 2023, Identification and Morph-metric Characterization of powdery mildew infecting diverse host plants of Southern Gujarat, India. *IJPSS*, **35** (20) : 153 - 166.

Perez Garcia, A., Romero, D., Fernandez Ortuno, D., Lopez Ruiz, F., De Vicente, A. and Tores, J. A., 2009, The powdery mildew fungus *Podosphaera fusca*

(synonym *Podosphaera xanthii*), a constant threat to cucurbits. *Mol. Plant Pathol*., **10** : 153 - 160.

Qasim, M., Akutse, K. S., Hussain, D., Al-Zoubi, O. M., Mustafa, T., Aguila, L. C. R., Alamri, S., Hashem, M., and Wang, L., 2022, Powdery mildew fungus *Oidium lycopersici* infected tomato plants attract the non-vector greenhouse whitefly, *Trialeurodes vaporariorum*, but seem to impair their development. *Agron.*, **11** : 2791.

Rur, M., Ramert, B., Hokeberg, M., Vetukuri, R. R., Grenville-Briggs, L. and Liljeroth, E., 2018, Screening of alternative products for integrated pest management of cucurbit powdery mildew in Sweden. *Eur. J. Plant Pathol*., **150** (1) : 127 - 138.

Smith, R. L., May, T. W., Kaur, J., Sawbridge, T., Mann, R., and Edwards, J., 2021, Molecular data from up to 130 year-old herbarium specimens do not support the presence of cherry powdery mildew in Australia. *Plant Pathol.*, **70** (3) : 689 - 698.

Swathi, B., 2022, Evaluation of cucumber (*cucumis sativus* L.) Genotypes for growth, yield and quality traits (Doctoral dissertation, University of Agricultural Sciences, Bangalore).

Takamatsu, S., 2018, Studies on the evolution and systematics of powdery mildew fungi. *J. Gen. Plant Pathol.*, **84** : 422 - 426.

Tamura, K., Stecher, G. and Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. *MBE.*, **38** (7) : 3022 - 3027.

Thompson, J. D., Higgins, D. G. and Gibson, T. J., 1994, ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*., **22** (22) : 4673 - 4680.

Wang, X. Z., Xu, B. Y., Ping, W., Luan, F. S., Ma, H. Y. and Ma, Y. Y., 2013, Identification of powdery mildew pathogen and ribosomal DNA-ITS sequence analysis on melon. *J. Northeast Agric. Univ.* (Engl. Ed.), **20** (4) : 10 - 18.