RESEARCH ARTICLE

# Characterization of Powdery Mildew Pathogen (*Erysiphe necator*) [Schw.Burr] Infecting Grapes in Tamil Nadu

**Veerathilagam D[1]*, Kannan R[1], Parthiban VK[1], Vincent S[2], and Bhuvaneswari K[3]**

[1]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore-641 003, India
[2]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore-641 003, India
[3]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, India

Received: 18 June 2021

Revised: 05 August 2022

Accepted: 04 September 2022

## ABSTRACT

Powdery mildew of grapes is one of the most criticalwidespread and destructive diseases worldwide, and it causes yield reduction upto 65-80 percent. In the present study, the survey was conducted in different districts of Tamil Nadu and percent disease index was assessed. The preliminary identification of the pathogen was madethrough phase contrast microscopic observation such as color, shape,and size of the conidia were measured as well as scanning electron microscopy was also used to study the morphometric characters of *E.necator*. Further,the isolates were confirmed through PCR analysis which yielded an amplicon size of 470 bp while using the specific primers (cyt b forward and cyt b reverse) targeting the *E.necator*.

**Keywords:** *Grapes; Powdery mildew; Erysiphe necator [Schw.Burr]; Survey; Morphology; Molecular characterization*

## INTRODUCTION

Grapes (*Vitis vinifera*) is an important fruit crop cultivated worldwide, mainly for vine manufacturing. Powdery mildew is one of the most important destructive diseases in grapes growing area poses a serious threat. It reduces the yield by up to 65 percent (Rao, 1992) and poses a serious threat to the wine industry. Grape powdery mildew pathogen is an obligate parasitic ascomycetous fungus that relies entirely on a host cell in photosynthesis-active tissues to complete its life cycle. Once conidia of powdery mildew lands on the epidermis of grape leaves, it germinates to form lobed appresorium. Later, the conidia can produce germ tubes to penetrate the plant tissues with the help of fungal lytic enzymes such as lipase, estrases, and cutinases. The typical symptoms of grapes powdery mildew fungus can be seen on all green tissues of the host plant affected (David *et al.,* 2012). Small, white, or grayish-white patches of fungal growth appear initially on the upper surface of the leaves. These patches usually enlarge until the entire upper leaf surface has a powdery, white-to-gray coating. Severely affected leaves may curl upward during hot, dry weather. Expanding leaves that are infected may become distorted and stunted. In berries, the severely affected fruit often split and open. In the present study, the identification of powdery mildew pathogen was observed through microscopic observation and confirmed through molecular-based detection techniques using Polymerase Chain Reaction (PCR) followed by sequencing.

## MATERIAL AND METHODS

### *Survey and collection of powdery mildew infected plant material*

A survey was conducted to assess the occurrence of powdery mildew disease in grapes-growing areas in Tamil Nadu. The powdery mildew-infected leaf samples were collected from various grapes-growing areas of Coimbatore and Theni districts. (Table 1) and the was calculated using the formula (1-9) proposed by Mickinney (1923).

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of numerical rating}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

The conidial spore mass extracted from the leaf samples was treated as different isolates and stored under -20˚C for further use.

### *Morphological characterization using phase contrast microscope and SEM*

The leaf samples expressing typical white powdery growth consisting mycelia, conidiophore and conidia were used for the study. Such leaf samples were gently scraped, transferred to a clean, sterile microscopic slide, and observed under the phase contrast microscope (Leica DM 2000 LED) . Powdery mildew conidiophore and conidia were photographed and conidial shape and size were measured.

*Corresponding author's e-mail: thilagamagri25@gmail.com

### *Scanning electron microscopy*

The powdery mildew-infected leaf samples were collected from the field and used for morphological analysis. The morphometric parameters of powdery mildew infected specimen were viewed under a scanning electron microscope (SEM: Quanta 250, FEI, Hillsboro., OR, USA) with a large field detector (LFD). The SEM was operated in vacuum (10KV) with a spot size of 3.0 and pressure of 60 Pa. The sample images were recorded at 1000 X, 5000 X, and 10000 X magnification. For the analysis of morphological characters, the powdery mildew-infected leaf samples were cut into small pieces with the help of a sterile razor blade. The size of the sample is approximately 5x5 mm. Then the live specimens were shifted directly into carbon stubs. The source of electron used in the SEM Tungsten filament and thermionic emission was used for detecting the samples using SEM.

### Molecular confirmation of powdery mildew pathogen

### *Extraction of genomic DNA from powdery mildew-infected grapes leaves*

The conidial DNA was extracted from the freshly collected grapes powdery mildew-infected leaf samples by using the modified CTAB method. The conidia were collected from powdery mildew-infected grapes leaves using a camel hair brush and transferred to a Micro centrifuge tube containing 300 µL of CTAB extraction buffer (50 mMTris- HCl, pH 8.0; 0.7 M NaCl and 1% CTAB (w/v) and vortexed for few minutes. The tubes were incubated at 60ºC for 20 minutes in a water bath. After incubation, an equal volume of phenol: chloroform: iso amylalcohol (25:24:1) the mixture was centrifuged at 13,000 rpm for 10 min. The aqueous layer was transferred to a fresh micro centrifuge tube, and the DNA was precipitated by adding an equal volume of ice-cold isopropanol and incubation at – 20ºC overnight. The DNA was collected by centrifugation at 13, 000 rpm for 10 min, discording the aqueous phase. The pellet was washed twice with 70 percent ethanol, air dried and was resuspended with 30 µL of nuclease-free water. The genomic DNA concentration was check edusing Nanodrop ND-3300 Fluoro spectrometer (NanoDrop products, Thermo Scientific, Wilmington, DE, USA).

### *Detection of Grapes powdery mildew through PCR*

The PCR assay was performed using a primer which is highly specific to *E.necator* including forward primer cytochrome b (cyt b) cyt b F (TGTTGTAATATTTATTTTAATG) and cyt b R reverse primer (TGGGTTAGCCATAATATAA). The PCR reaction mixture consisted of a 5µL of 2x master mix 1 µL of forward and reverse primer, 1 µL of genomic DNA (50ng/µL), and 2 µL of nuclease-free water. The reaction was carried out using the PCR conditions as follows, initial denaturation of 3 min at 94˚C followed by 40 cycles of denaturation at 94˚C for 30 seconds, annealing at 42˚C for 1 min, extension at 72˚C at 90 seconds and final extension at 72˚C for 7 min. Finally, the amplified products were confirmed through gel electrophoresis in 1 % agarose containing ethidium bromide (2 µL) at 80 V for I h and documented in gel documentation unit (UVITECH, Cambridge).

## RESULTS AND DISCUSSION

In this study, the cultural, morphological, and molecular characterization of *E. necator* infecting grapes was carried out. Grapes powdery mildew is an important destructive disease causes a yield reduction of 70 percent (Kong *et al.,* 2016) to 80 percent (Falacy *et al.,* 2007) respectively. Gadoury *et al.* (2011) reported that the powdery mildew symptoms were observed in all green parts of the grapes. A specific characteristic the Erysiphales members is the production of mycelium superficially on the host surface. Consequently, the formation of appresoria is obligatory to ensure direct host penetration and further infectious development (Emmett *et al.,* 1975).

### *Survey and collection of powdery mildew-infected grapes samples*

The powdery mildew disease was observed in all green tissues of grapes like leaves, stems, inflorescence, and berries. On leaves, the initial infection of powdery mildew appeared as small dull whitish patches on the upper surface of the leaves (Fig 1) later, the patches enlarged and covered a large proportion of the leaf blade. The severely infected fruits also exhibited cracks. The survey was conducted in major grapes-growing areas in Tamilnadu. Viz., Cumbum, Thondamuthur, TNAU orchard, Karadimadai, Mathipalayam, Surulipatty, Chinnaovalapuram, and Anaimalayanpatty. The highest disease severity was recorded in Cumbum (67.04 %) followed by Mathipalayam 65.31 per cent (Figure 2). These results were found in accordance with Karthik *et al.* (2019), who surveyed the grapes growing areas in different parts of Tamilnadu, and observed the highest powdery mildew disease severity at Cumbum (63.38 %) followed by Kamayagoundanpatti ( 60.76 %).

### *Morphological characterization*

The powdery mildew-infected grapes leaf samples were collected from different locations in Coimbatore and Theni districts. Samples were subjected to morphological observation under phase contract microscopy at 40X magnification. The conidiophore was multiseptate in nature and bore conidia.

The conidia were hyaline and ovoid in shape (Figure 3). The largest size of conidium was observed in Cumbum isolate, (36.53 µm length and 15.48 µm breadth) followed by Karadimadai isolate 35.49 µm

length and 15.29 µm breadth (Table 1). Similarly, Gadourly *et al.* (2011) studied the morphological character of grapes powdery mildew and reported the length (44.22-45.96 µm) and breadth (11.56-12.26 µm) of the conidia of *E.necator*. Similarly, Karthik *et al.* (2019) studied the morphological characteristics of *E.necator* infecting grapes powdery mildew in India and reported the size of conidia as 31.74-36.43 µm length and 12.85-15.45 µm breadth.

Figure 1. The powdery mildew infection of *E. necator* infected in all living tissues of the grapes plant. A whitish patches present on adaxial surface of the leaves. B.The fungal mycelial colonies covered entire young berries. The severely infected berries become split and open.

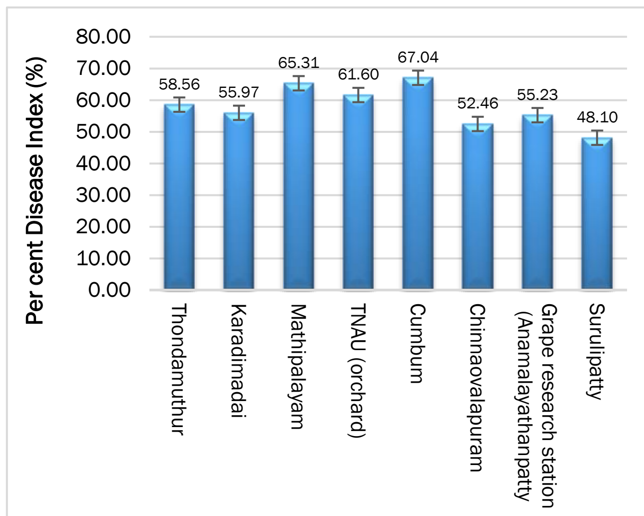


Figure 2. Survey and collection of grapevine powdery mildew infected samples from different parts of leaf samples from different and calculate the disease severity

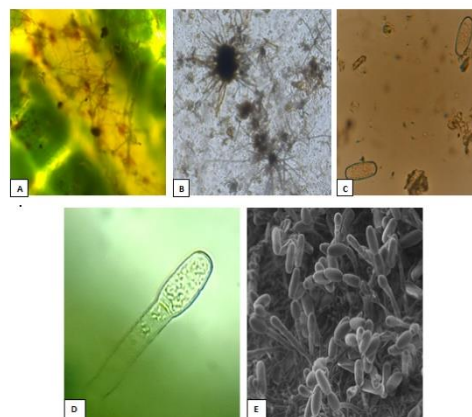


Figure 3. Microscopic observation of powdery mildew infected grapes leaves through phase contract microscopy at 40 X magnification. A. An intercalary

**Table 1. Morphological characters of *E. necator* conidia at 40x magnification**

| S.No. | Place | District | Colour of the conidia | Shape of the conidia | Length(µm) | Breadth(µm) |
|---|---|---|---|---|---|---|
| 1. | Thondamuthur | Coimbatore | Hyaline | Ovoid to barrel shape | 31.76 | 12.87 |
| 2. | Karadimadai | Coimbatore | Hyaline | Ovoid to barrel shape | 35.49 | 15.29 |
| 3. | Mathipalayam | Coimbatore | Hyaline | Ovoid to barrel shape | 32.91 | 13.11 |
| 4. | TNAU (orchard) | Coimbatore | Hyaline | Ovoid to barrel shape | 33.25 | 13.81 |
| 5. | Cumbum | Theni | Hyaline | Ovoid to barrel shape | 36.53 | 15.48 |
| 6. | Chinnaovalapuram | Theni | Hyaline | Ovoid to barrel shape | 32.33 | 12.53 |
| 7. | Grape research station (Anamalayathanpatty) | Theni | Hyaline | Ovoid to barrel shape | 33.81 | 14.75 |
| 8. | Surulipatty | Theni | Hyaline | Ovoid to barrel shape | 31.94 | 12.92 |

*Scanning electron microscopy studies of powdery mildew pathogen*

In this study, the ultrastructural and morphology characterization of *E. necator* revealed the development stages of *E. necator* present in host tissues. The chains of conidia can be seen on the conidiophore on upper surface of the leaves (Fig 4). The conidia become hyaline and cylindro-ovoid in shape. In this present study, the morphometric parameters of the pathogen present in the infected leaves, such as conidiophore bearing single conidia were observed under SEM. The findings were found similar to the earlier report of Ki Woom Kim (2021), who also studied the difference between *E. necator*-infected grapes leaves and healthy leaves and demonstrated the morphology and infection- related characteristics of *E.necator* through field emission scanning electron microscopy.

*PCR amplification of E.necator*

PCR detection of grapes powdery mildew pathogen *E.necator* infected leaves as used to extract DNA and subjected to PCR amplification. Species specific primers cyt b forward and cyt b reverse were used to target the *E.necator*. The PCR reaction yielded an amplicon size of 470 bp. (Fig 5). The amplicons were sequenced, and the sequences were deposited in the NCBI database under the accession number PM1 ON566016, PM 2 ON 566125, PM 3 ON566202, and PM 4 ON568586. The confirmation study was carried out by molecular characterization through PCR techniques. The PCR analysis yielded 470 bpamplicon that confirmed the presence of *E.necator*. This agrees with the results of Karthik *et al.* (2019) and Basha *et al.* (2021).

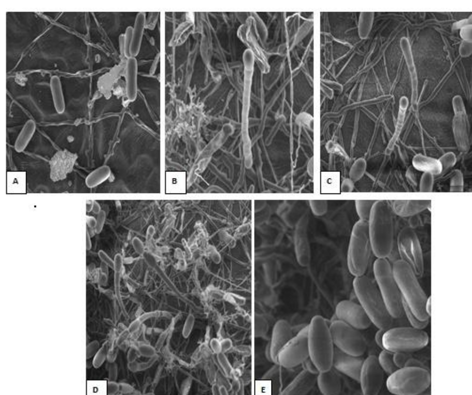


**Figure 4. Scanning electron micrographs of powdery mildew infected grapes leaves. A. formation of appressorium.B. Conidiophore, C. Conidiophore consisting multi septate and immature conidia at the end. D. The rounded and cylindro-ovoid shape conidia present on the distal end of the condidiophore. E. Mass of individual conidia**

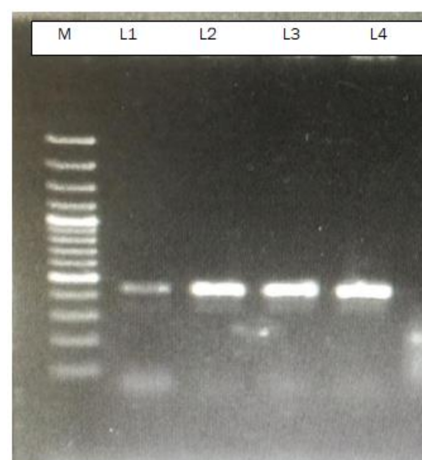


**Figure 5. PCR amplification of *E.necator* using specific gene cyt b DNA isolated from powdery infected grapes leaves. M-ladder, L1-TNAU grapes orchard, L2- Karadimadai, L3- Cumbum L4- Thondamuthur, and L5 Negative control (Nuclease free water)**

## CONCLUSION

The powdery mildew disease severity was assessed in different grapes growing areas. And the pathogen was Identified through microscopic observation and confirmed the pathogen by using the molecular characterization of *E. necator*.

## ACKNOWLEDGEMENTS

The authors acknowledge the Department of Plant Pathology, Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

*Ethics statement*

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

*Originality and plagiarism*

Authors should ensure that they have written and submit only entirely original works, and if they have used the work and/or words of others, that this has been appropriately cited. Plagiarism in all its forms constitutes unethical publishing behavior and is unacceptable.

*Consent for publication*

All the authors agreed to publish the content.

*Competing interests*

There were no conflict of interest in the publication of this content

*Data availability*

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; thilagamagri25@gmail.com

*Author contributions*

Research grant-R.K, Idea conceptuation-R.K, Experiments-V.D, R.K, V.K.P., Guidance- , R.K, V.K.P, S.V,K.B Writing original draft- D.V,R.K, V.K.P, S.V,K.B Writing-reviewing & editing R.K, V.K.P, S.V,K.B.

## REFERENCES


Alimad, N., Naffaa, W. and Azmeh, F., 2017.Overwintering form of *Erysiphe necator*, the causal agent of grapevine powdery mildew in southern Syria. *Journal of Plant Protection Research*, **57(2).**

Falacy JS, Grove GG, Mahaffee WF, Galloway H, Glawe DA, Larsen RC, Vandemark GJ. Detection of *Erysiphe necator* in air samples using the polymerase chain reaction and species-specific primers. *Phytopathology*. **2007;97(10):**1290-7. 16.

Gadoury, D.M., CADLE-DAVIDSON, L.A.N.C.E., Wilcox, W.F., Dry, I.B., Seem, R.C. and Milgroom, M.G., 2012. Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Molecular plant pathology*, **13(1),** pp.1-16.

Gadoury, D.M., Wilcox, W.F., Rumbolz, J. and Gubler, W.D. (2011) Powdery mildew. In: Compendium of Grapevine Diseases ( 2nd edn.) ( W.F. Wilcox, W.D. Gubler and J. Uyemoto, eds). APS Press (in press).

Karthick, M., Kamalakannan, A., Malathi, V.G., Paranidharan, V., Sivakumar, U., Kavino, M. and Gowrisri, N., 2019.Phenotypic Characterization and Molecular Phylogenetic Relationship of *Erysiphe necator* Infecting Grapes (*Vitis vinifera*). *Current Journal of Applied Science and Technology,* pp.1-10.

Kim, K.W., 2021. Morphology and surface characteristics of the anamorphic stage of powdery mildew *Erysiphe australiana* on crape myrtle leaves. Micron, **143,** p.103013.

Kong X, Qin W, Huang X, Kong F, Schoen CD, Feng J, Wang Z, Zhang H. Development and application of loop mediated isothermal amplification (LAMP) for detection of *Plasmo paraviticola*. Scientific reports. 2016;**6(1):**1-9.

Qiu W, Feechan A, Dry I. Current understanding of grapevine defense mechanisms against the biotrophic fungus (*Erysiphe necator*), the causal agent of powdery mildew disease. *Horticulture research.* 2015 Dec 23;2.

Rao KC. Epidemiology of some common diseases of grape around Hyderabad. In Proceedings of International Symposium on Recent Advances in Viticulture and Oenology, Hyderabad, India 1992; **323329.**

Shajith Basha, J., Kamalakannan, A., Saraswathy, S., Johnson, I., SantoshGanapati, P. and R Swarna Lakshmi, K., 2021. Rapid Detection of Airborne Inocula of Grapevine Mildews Using PCR and LAMP Assay.