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Morphological characterization of *Plasmopara viticola*, the inciting agent of grapes downy mildew

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Abstract
Grapes downy mildew is incited by the most destructive pathogen called *P. viticola* is a major bottleneck for grapevine production. The morphological and ultra structural analysis of the pathogen plays a significant role in order to take precautionary management strategies. For morphometric analysis the downy mildew infected leaf samples were collected from eight different locations in Coimbatore and Theni districts. The collected samples were subjected to morphoplogical analysis under light microscope at 40 X magnification. The length and breadth of the sporangia vary from 19.94 - 22.73 µm and 14.58-17.69 µm. The scanning electron microscopic analysis of *P. viticola* shows the developmental stages of the pathogen on the host leaf surface. The Pathogenicity of the pathogen was confirmed through detached leaf assay. The characteristic sporulation of the fungus was observed at 7th day after post inoculation.

Keywords: Downy mildew, *Plasmopara viticola*, morphological, scanning electron microscopy

Introduction
Grapes is widely cultivated all around the world mainly for fruits, juice and manufacturing of wines. The practice of growing grapes over larger area is threatened by most destructive disease called downy mildew (Rumbolz *et al.* 2002) [8]. Downy mildew is one of the most vulnerable one which reducing the yield of grapes and poses a serious threat to wine industry.
The grapes downy mildew disease is incited by obligate oomycetous parasitic pathogen *Plasmopara viticola*. It causes extensive yield loss by affecting all the green tissues of grapevine including leaves, inflorescence and clusters (Choi *et al*. 2017) [2]. The *P. viticola* survives as oospores in dead leaves during winter period. The oospores act as primary source of inoculum for initiating the disease. Oospores forms a germ tube inorder to form sporangia. The sporangia containing mass of zoospores which are released at the time of desiccation of sporangia.
The released zoospores survive on host tissue more than 24 hrs under cool climatic condition. Sporangia act as secondary spread of the pathogen and are dispersed through rain splash and wind. For the formation of sporangiophore and sporangia, it requires 95-100% RH and temperature level of 13ºC. The zoospores released from sporangia swim in free water in host tissue, they encyst and germinate. The germinated zoospores penetrate stomata through germ tube. At the end of the season, oospores were formed on the infected host tissue. The temperature level of above 10ºC or atleast 10mm rainfall level necessary for oospore germination in wet soil (Kennelly *et al*., 2007) [3]. For the appearance of visible symptom in the host, the incubation period vary from five to twenty one days (Rossi *et al*., 2013; Caffi *et al*., 2013) [7, 1].
The infected downy mildew leaves will turn yellow or paler green with angular spots on the upper surface and whitish fungal growth on lower side of leaves. As the disease progress, the infected leaves shows necrotic lessions (Choi *et al*. 2017) [2]. The sporangiophore will emerge out of stomata. The sporangiophores were hyaline, straight or slightly curved and right angle branched. Sporangia were hyaline oval or lemon shaped and produced on right angle sporangiophore. The sporangium contains flagellate zoospores which will be released upon contact with water (Choi *et al*., 2017) [2].
Considering the importance of downy mildew infection and their potentiality of being an epidemic pathogen, morphological and ultra structure analysis will be helpful in managing the disease much earlier. In this present study, the morphological characterization of downy mildew pathogen along with ultra structural analysis of *P. viticola* through scanning electron microscopy was done as an initiative step for effective management of the disease.

Materials and Methods

Morphological characterization of *P. viticola* isolates collected from different places

The grapes downy mildew infected leaf samples were collected from eight different locations in Coimbatore and Theni districts. The leaf samples expressing the typical downy mildew symptom of oily yellow spot on upper leaf surface and velvety downy growth on lower surface were thin sectioned and observed under light microscope. Three samples were observed for each location and totally twenty four samples from eight different locations were analyzed. For morphometric analysis fifty sporangia were observed and their size including length and breadth of the sporangia was measured at 40X magnification. Finally *P. viticola* sporangiophore and sporangia were imaged using a Labomed camera model LX400 with an image analyzer pixel pro program.

Scanning electron microscopy image of *P. viticola*

The downy mildew infected grapevine leaf sample collected from the field were used for morphological analysis using Environmental scanning electron microscopy (ESEM) analysis. The morphometric parameters of downy mildew infected specimen were viewed using a scanning electron microscopy (SEM: Quanta 250, FEI, Hillsboro, OR, USA) with an Large Field Detector (LFD). The SEM was operated in vacuum 10KV with a spot size of 3.0 and a pressure of 60 Pa. The sample images were recorded at 5000 X and 10000 X magnification. For analysis of morphological characters the leaf material infected with *Plasmopara viticola* was hand cut with a razor blade into small pieces of approximately 5 x 5 mm. Then the live specimens were fixed directly in carbon stubs. The source of electron used in the ESEM was Tungsten Filament and Thermionic emission was used for the detection of the samples using ESEM.

Pathogenicity of *P. viticola* in grapes

The pathogencity of *P. viticola* on grapes was proved by procedure described by Li *et al*., (2016) [5] with slight modification. Downy mildew infected grapevine leaf samples were collected from farmers field and kept in a ziplock plastic cover in an ice box and transferred to the laboratory condition. The *P. viticola* infected leaf samples were placed on 0.7% agar and incubated under growth chamber for 22 ^{0}C at overnight in order to artificially induce the sporulation of the pathogen. After sporulation the sporangial concentration was adjusted to 1 X10^4 sporangia/ml. For proving pathogenicity of the pathogen healthy leaves were collected from glass house grown grape variety, Paneer. The detached leaves were placed on the 0.7% agar with abaxial layer in upper side. Excess water in the leaf sample was removed by sterile filter paper. The 30µl of sporangial suspension was drooped on the lower leaf surface of grapevine. Petri plates were incubated at 22 ^{0}C under growth chamber of 16 hrs of light and 8 hrs of dark period in order to favor the sporulation. Formation of white fungal growth of the pathogen on the lower surface of leaves were observed 7 days after post inoculation. The leaf sample without inoculation of the pathogen was kept as control. Totally three replication were maintained.

Result

Morphological characterization

The downy mildew infected leaf samples collected from were eight different locations in Coimbatore and Theni districts were subjected to morphological analysis. The sporangiophore and sporangia of the fungus was observed at 40X magnification in light microscope. The sporangia were hyaline, oval shaped formed on right angle sporangiophore. The sporangiophore were hyaline, straight or slightly curved and right angle branched. The length and breadth of the sporangia varied from 19.94 - 22.73 µm and 14.58-17.69 µm respectively. The largest size of the sporangia was observed in Kamayagoundanpatti with a average length of 22. 73 and breath of 16.79 µm, followed by TNAU Coimbatore isolate having average size of 22.21 and 16.46 µm in length and breadth respectively. The lowest size of sporangia were observed in Mathipatty isolate having a length and breadth of 19.71 and 14.28 µm (Fig 1; Table 1).

Scanning electron microscopy image of *P. viticola*

The ultra structural study of interaction between *P. viticola* revealed that the developmental stages of *P. viticola* on host tissue. Initially, zoospores will attach to the lower leaf surface from desiccated sporangia, it will leads to systemic mycelial colonization inside the host tissue. The right sporangiophore were emerge nced from stomata of infected leaf. The sporangia were hyaline, oval or lemon shaped formed on tip of the right angle sporangiophores (Fig 2).

Pathogenicity of *P. viticola*

The pathogenicity *P. viticola* (TNAU isolate) were performed by inoculation of sporangia on lower leaf surface in 0.7% water agar. The whitish sporulation of the fungus was observed on lower leaf surface at 7th day after inoculation. Whitish fungal growth of the pathogen consisting of mass of sporangia on right angle sporangiophores, which is the typical character of the fungi. It shows that symptom expression is similar to that of natural of *P. viticola* in grapes, which confirms the pathogenicity nature of *P. viticola* (Fig 3).

Discussion

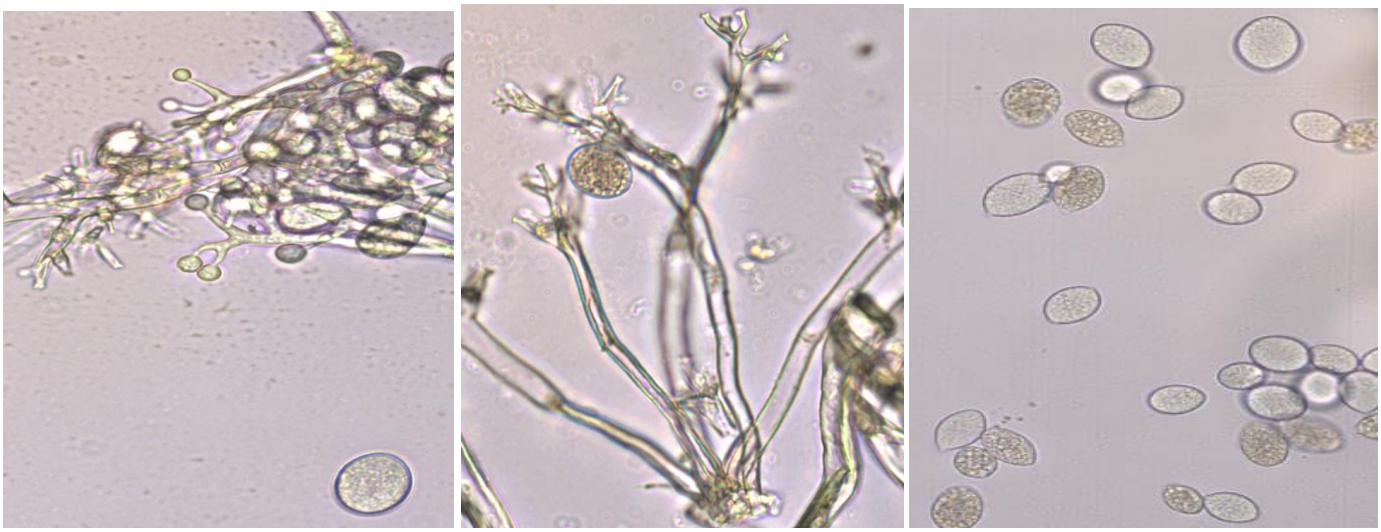
In this study, the morphological characterization of *P. viticola* infecting grapes was deeply studied. Further the infective nature of the pathogen was clearly studied by pathogenicity test. The ultra structure identification and analysis of infected leaves revealed the infection process of *P. viticola*, thereby the improvement of breeding strategies for management of downy mildew disease can be done in near future.

The morphology of *P. viticola* was studied through light microscope at 40 X magnification. The length and breadth of the sporangia of eight different isolates varied from 19.94 - 22.73 µm and 14.58-17.69 µm respectively. Similarly Kim *et al*., (2019) [4] observed the morphology of *P. viticola* in grapes. They reported that sporangia were lemon shaped and the size varied from 20.9-24.4 and 13.9 – 16.6 µm in length and width. Choi *et al*., (2017) [2] studied the morphological features of *P. viticola* infecting grapes in Korea. They reported that, the size of the sporangia varied from 18.2-22.8 X 13.5 – 15.5 µm.

The ultrastructural study of interaction between *P. viticola* and grapes was studied through scanning electron microscopic analysis. The developmental stages of the pathogen was observed like zoospore attachment, emergence of sporangiophore from stomata, formation of oval sporangia on right angle sporangiophore. Similarly the Ma *et al*. (2018) [6] studied the infectious nature of *P. viticola* in grapes through SEM analysis. The series of events like zoospore attachment, sporangiophore emergence and formation of ovoid sporangia on right angle sporangiophore were observed.

Our results were also positively correlated with study of yin *et al*. (2007) [10].

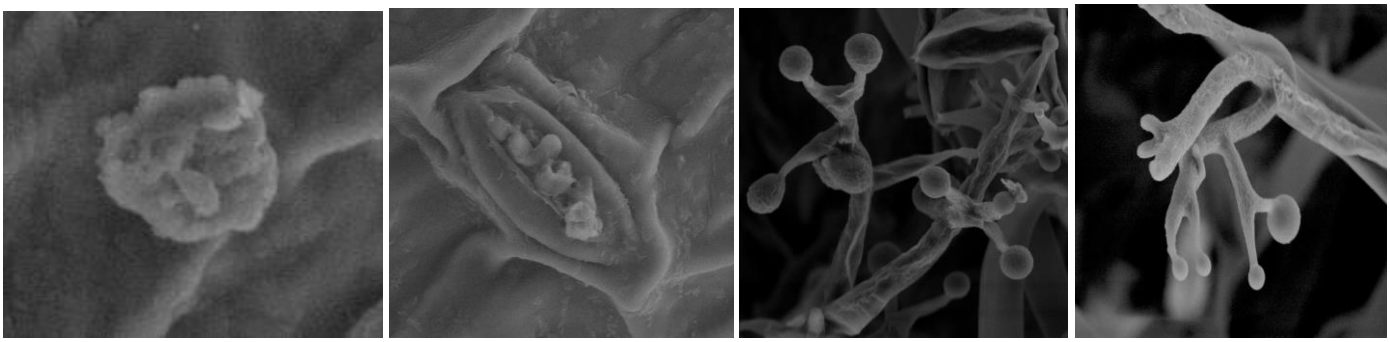
The infective nature of the pathogen was studied using pathogenicity test through detached leaf assay. The inoculated leaf expresses the characteristics sporulation of the fungus after 7th after inoculation. Similarly, Toffolatti *et al.* (2016) [9] proved the pathogenicity of *P. viticola* in different cultivars through detached leaf assay. They reported that downy mildew symptoms were expressed on different accessions vary from 7-10 days after post inoculation. Zeledon *et al.*, (2016) [11] proved the pathogenic ability of *P. viticola* by inoculating the sporangia on detached leaves. They reported that, the infection occurs after 10 day after post inoculation.



a. b. c.

Fig 1: Morphological characterization of *P. viticola* in light microscope at 40 X magnification

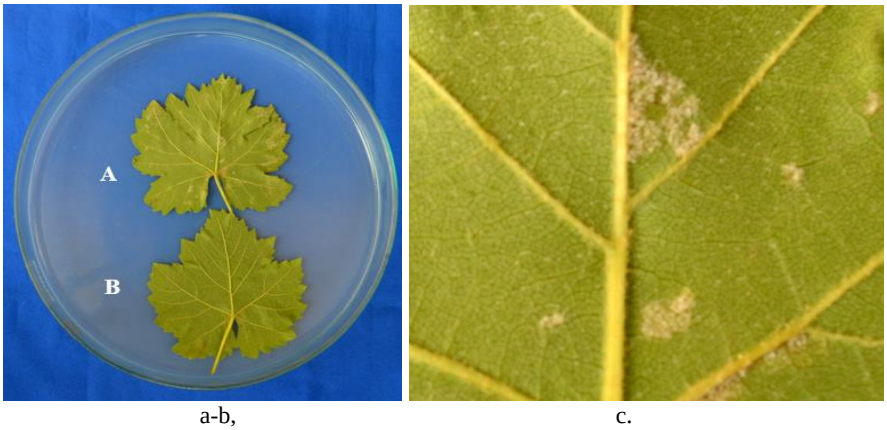
Light microscopic observation of *P. viticola* in light microscope at 40 X magnification. A) The development of immature sporangia on sporangiophore at initial stage. B) Development of matured sporangia on right sporangiophore at later stage. C) Group of sporangia shows hyaline and oval in shape



a. b. c. d.

Fig 2: Ultra structural study of interaction between *P. viticola* and grapes through scanning electron microscopy

Scanning electron microscopic analysis of *P. viticola* in grapes. A) Attachment of zoospores on lower leaf surface at initial stage of infection B) The emergence of sporangophore from stomata of the infected leaf C) Formation of sporangia on right angle sporangiophore at early stage D) Formation of matured sporangia on sporangiophore at later stage.



a-b, c.

Fig 3: Pathogenicity of *P. viticola* in grapevine leaf through detached leaf assay

Pathogenicity of grapes downy mildew in 0.7% agar at 7th day after inoculation A) Grapevine leaf Inoculated with sporangia of *P. viticola* B) Control (without inculation of *P. viticola*) C) Closer view of sporulation of *P. viticola* consisting of sporangiophore and sporangia (Inoculated).

Table 1: Microscopic observation of *P. viticola* sporangia at 40 X magnification

S. No	Place	District	Length (µm)	Breadth (µm)
1.	TNAU	Coimbatore	22.21	16.46
2.	Mathipatty	Coimbatore	19.71	14.28
3.	Grape research station (Anamalayapatty)	Theni	20.48	15.68
4.	Kamayagoundanpatti	Theni	22.73	16.79
5.	Rayappanpatty	Theni	21.47	16.23
6.	Anaipatty	Theni	20.19	15.30
7.	Surulipatty	Theni	19.94	14.58
8.	Cumbum	Theni	20.39	15.47

Reference

1. Caffi T, Gilardi G, Monchiero M, Rossi V. Production and release of asexual sporangia in *Plasmopara viticola*. Phytopathology. 2013; 103:64-73.
2. Choi YJ, Cho SE, Shin HD. First Report of Downy Mildew Caused by *Plasmopara viticola* on *Vitis ficifolia* var. *Sinuata* in Korea. Plant Disease First Look paper. Plant disease. 2017; 101(11).
3. Kennelly MM, Gadoury DM, Wilcox WF, Magarey PA, Seem RC. Primary infection, lesion productivity and survival of sporangia in the grapevine downy mildew pathogen Plasmopara viticola. Phytopathology. 2007; 97:512-522.
4. Kim BR, Kim IH, Lee JS, Choi YJ. First Report of Downy Mildew Caused by *Plasmopara viticola* on *Vitis coignetiae* in Korea. Plant disease. 2019; 103(7)
5. Li X, Yin L, Ma L, Zhang Y, An Y, Lu J. Pathogenicity Variation and Population Genetic Structure of *Plasmopara viticola* in China. Journal of phytopathology. 2016; 164(11-12):863-873.
6. Ma H, Xiang G, Li Z, Wang Y, Dou M, Su L *et al*. Grapevine VpPR10.1 functions in resistance to *Plasmopara viticola* through triggering a cell death-like defence response by interaction with VpVDAC3. Plant Biotechnol J. 2018; 16(8):1488-1501.
7. Rossi V, Caffia T, Gobbin D. Contribution of molecular studies to botanical epidemiology and disease modelling: grapevine downy mildew as a case-study. European Journal of Plant Pathology. 2013; 135(4):641-654.
8. Rumbolz J, Wirtz S, Kassemeyer HH, Guggenheim R, Schafer E, Buche. Sporulation of *Plasmopara viticola*: Differentiation and Light regulation. Plant biology. 4(3):413-422.
9. Toffolatti SL, Maddalena G, Salomoni D, Maghradze D, Bianco PA, Failla O. Evidence of resistance to the downy mildew agent *Plasmopara viticola* in the Georgian *Vitis vinifera* germplasm. Vitis. 2002-2016; 55:121-128.
10. Yin X, Liu R, Su H, Su L, Guo Y, Wang Z *et al*. Pathogen development and host responses to *Plasmopara viticola* in resistant and susceptible grapevines: an ultra-structural study. Horticulture Research. 2017; 4:17033.
11. Zeledon JG, Kaiser M, Spring O. An Extended Leaf Disc Test for Virulence Assessment in Plasmopara viticola and Detection of Downy Mildew Resistance in Vitis. J Plant Pathol Microbiol, 2016, 7(5).