

MORPHOLOGICAL VARIATION IN *Hemileia vastatrix* CAUSING COFFEE LEAF RUST FROM COFFEE GROWING REGIONS IN SOUTHERN KARNATAKA, INDIA

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Abstract

Throughout the world, coffee is an important agricultural commodity and most consuming beverages. Over the last two decades, coffee production has been declined due to the outbreak of the most devastating disease called coffee leaf rust caused by *Hemileia vastatrix*. To achieve sustainability, soil biodiversity plays a key role in the agriculture system as the indicator of soil health. A total of 29 different localities were surveyed for coffee leaf rust disease in major coffee-growing regions such as Chikkamagaluru, Kodagu, and Hassan in Karnataka, India. The present study reveals the morphological variation of *Hemileia vastatrix* causing coffee leaf rust disease from different geographical regions, observed under a light microscope and scanning electron microscope

Key words: coffee leaf rust, urediniospores, Scanning Electron Microscope, disease severity, morphological variation.

INTRODUCTION

Coffee is a vital crop and beverage worldwide, creating incomes for over 125 million people. In terms of commerce, it is the second most exported commodity after oil. Coffee production is a crucial aspect of the global economy, with main producers including Brazil, Vietnam, Colombia, and Indonesia. The coffee plant is intuitive to the subtropical region of Africa and some islands of Southern Asia. Brazil is the largest coffee producer globally, responsible for 40% of the world's total production, with 69.9 million bags in 2021. Other major coffee-producing countries include Vietnam, Colombia, and Indonesia. In South India, Karnataka accounts for 71% of the total coffee production, followed by Kerala (21%) and Tamil Nadu (5%). (Rossi Moda et al., 2022; Vijayan et al., 2022; Silva et al., 2023; Soares et al., 2023). The coffee production regions in Karnataka include Chikkamagaluru, Kodagu, and Hassan. Chikkamagaluru, known as the Coffee Land of Karnataka, is a primary location for *arabica* coffee cultivation (Nayak et al., 2023). Additionally, coffee is mainly grown in

Kodagu and Hassan districts, part of the Western Ghats in Karnataka (Reddy et al., 2022). Traditionally, coffee being grown in tropical and subtropical regions, belongs to the Rubiaceae family and the genus *Coffea* which consists of 90 to 124 species (Silva et al., 2022). Coffee being a perennial crop is continuously affected by many pathogens under unfavourable conditions (Wellman, 1953; Waller, 1982). Over the last two decades, coffee production has been declined as compared to consumption which has been on a steady expansion over the same period. Because of climate change, the production of coffee has been negatively affected which directly affects yield and quality (Pham et al., 2019). Coffee production has been wiped out due to coffee leaf rust disease in the past 150 years and still causing serious issues across coffee-growing regions (Talhinhas et al., 2017). The decline in the production of coffee due to coffee leaf rust which is one of the devastating diseases caused by *Hemileia vastatrix* Berk & Broome affecting coffee crops worldwide. In *arabica* variety coffee leaf rust is a major disease, causing substantial economic loss in more than 50 countries. The fungal disease is hosting specific causes severe losses

of berries and foliage up to 70-75% (Rutherford & Phiri, 2006; McCook, 2006; Suresh et al., 2012).

Hemileia vastatrix is an obligate parasite, that deposits on the lower side of the coffee leaf and colonizes intercellular to complete their reproductive cycle until their host is alive. The symptoms of CLR (coffee leaf rust) form the chlorotic spots in leaves, reduce photosynthetic rate, defoliation, and decrease quality and quantity of coffee production. Spore germination of *Hemileia vastatrix* is favoured by a dark humid environment between 21°C-27°C and available water on the leaves up to 8 h. The process of infection begins at the penetration phase of *H. vastatrix* spores after the adhesion to the underside leaf surface, a crucial phase for the process of pathogenesis since it prevents the displacement of pathogens from their host and enhances thigmotropism. Soon after the first contact, the germination tube is formed in the dikaryotic phase, the end of the germination tube has a hypha hook like tip that senses and transduces the leaf surface via signal chain, and the appressorium is formed after the stomata identification (Voegele et al., 2009; Castillo et al., 2022; RoyChowdhury et al., 2022).

Before the sporulation, small chlorotic spots may appear as young lesions. On the underside of leaves yellow to orange powdery blotches appear, that forms chlorotic patches on the upper side of leaves. These steadily expand up to 3-4 mm in diameter. Older leaves show several lesions and together produce irregular disease areas. The *Hemileia* is a genus belonging to the phylum Basidiomycota, class Pucciniomycetes, order Pucciniales (rust fungi) described by Berkeley & Broome (1869).

Hemileia vastatrix is a hemicyclic, obligate biotroph fungi which means that it depends on another living host for its growth and reproduction, and also produces urediniospores, teliospores, and basidiospores, but only the dikaryotic urediniospores, which form the asexual part of the cycle, reinfect coffee leaves frequently and are responsible for the disease proliferation. The reproductive cycle of rust fungi includes two parasitic stages, dikaryotic and monokaryotic. A significant impact on coffee production has been observed, during severe rust infection leads to the premature fall of the leaf, which reduces plant photosynthesis

area debilitates the plant, and can cause branch dieback (Sera et al., 2022). During favourable environmental conditions, the urediniospores reinfect the leaves which are dikaryotic and represent the asexual cycle. However, at different times, urediniospores and teliospores are produced in the same sorus. Teliospores produce a promycelium which forms four basidiospores. Basidiospores of *Hemileia vastatrix* do not infect coffee plants (Kushalappa & Eskes, 1989; Talhinas et al., 2017). Thus, the current study aims to analyse morphological variation in *Hemileia vastatrix* urediniospore which causes coffee leaf rust, the most devastating disease seen in coffee-growing regions, examining the disease severity of *Hemileia vastatrix* and macroscopic and microscopic identification of isolated fungi from soil. Subsequently, study investigates the potential spread of coffee leaf rust disease.

MATERIALS AND METHODS

Study location

Karnataka state lies between longitudes 74°12'00" to 78°41'00" E and latitude 11°31'00" to 18°45'00" N and seventh largest state in India. The field survey was done to know the severity of coffee leaf rust disease in major coffee growing regions of Karnataka: Chikkamagaluru, Hassan, and Kodagu. These areas lie between 75°28'00" to 75°45'00" E longitudes and 12°30'00" to 13°22'00" N latitudes and elevation between 920 to 1000 AMSL. A zone of transition between the Deccan Plateau and the Western Ghats is formed by the physiography (Figure 1; Table 1).

Collection of diseased plant samples

Collection of coffee leaf rust disease samples was done in 29 different locality of three districts. Disease sample was randomly monitored. Total ten leaves per tree were collected randomly from adjacent branches exhibiting a fungal pustules, were collected aseptically in ziplock polythene bags and brought to the laboratory and stored in cryopreservation at (-196°C) for further analysis of disease samples. Morphological variation of *Hemileia vastatrix* uredospore and teliospores was studied using CX23 Olympus light microscope (Alhudaib & Ismail, 2024) (Figure 2).

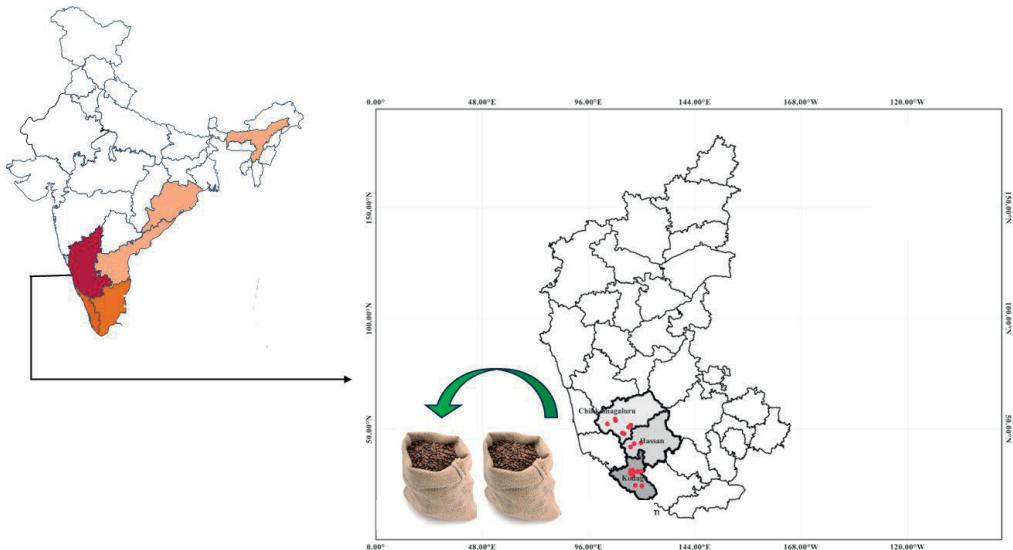


Figure 1. Field survey for coffee leaf rust disease was conducted in twenty-nine place of three districts of coffee growing regions of Karnataka



Figure 2. (A) Severity of Coffee leaf rust disease in coffee field, (B) Light microscopic image of *Hemileia vastatrix* spores under 40X magnification

Statistical analysis

The analyses CLR incidence in various elevation and disease severity index were performed in Microsoft excel using formula (Rhiney et al., 2021).

$$AI \% \text{ in lot} = \frac{\text{Number of disease leaves in 60 trees}}{\text{Total number of leaves in 60 trees}} \times 100$$

Table 1. Survey for coffee leaf rust disease in agro-climatic zone of in coffee growing regions of Southern Karnataka, India

District	Taluk	Place of collection	Coffee varieties grown in Karnataka
Chikmagalur	Sringeri	Sringeri	Arabica
	Narasimharajapura	B. Kanubur	Arabica
		Balehonnur	Robusta
	Chikmagalur	Aladagudde	Arabica
		Chikmagalur	Chandragiri
		Mahaji	Robusta
		Chithavalli	Arabica
	Mudigeri	Mudigeri	Robusta
		Baggasagodu	Arabica
Hassan	Hassan	Hassan	Robusta
	Arkalgud	Bychanahalli	Arabica
	Sakleshpura	Heggadde	Arabica
	Alur	Rangenahalli	Arabica
		Sakleshpura	Cavery
Kodagu	Madikeri	Madikeri	Robusta
		Katakeri	Arabica
		Hebbettageri	Arabica
		Hakathur	Arabica
		Ibnivalvadi Rural	Arabica
	Virajpet	Virajpet	Chandragiri
	Somwarpet	Basavanahalli	Robusta
		Kodagarahalli	Arabica
		Guddehosur	Arabica
		Andagove	Robusta
	Ponnampet	Ponnampet	Arabica
		Gonikoppal	Arabica
	Kushalnagar	Kushalnagar	Arabica
		7th Hosakote	Arabica
		Suntikoppa	Arabica

Analysis of *Hemileia vastatrix* spore by scanning electron microscope

Diseased leaf samples were mainly collected from 29 places in coffee-growing regions from Chikkamagaluru, Kodagu, and Hassan. All selected leaves of coffee varieties show the colonies of *Hemileia vastatrix* with the development of teliospores. SEM examination was done by fragmenting the fresh coffee leaves bearing the symptoms of coffee leaf rust, round discs about $0.5 \times 0.5 \text{ cm}^2$ containing spores and mycelia. The disc was mounted on aluminum stubs, using double-stick carbon tape. Samples were then dried in a critical point dryer (BAL-TEC model CPD 030) using liquid CO_2 as transition fluid and examined using a Zeiss EVO18 model (Fernandes et al., 2009; Gómez-de la Cruz et al., 2022).

Spore size of *Hemileia vastatrix* urediniospores

Total 29 selected diseased leaf samples were collected and individual spores were removed

from lower surface using sharp needle and observed under light microscope for urediniospore measurement (μm). Urediniospores per leaf was measured (CX23 Olympus). Wall thickness width, and length of urediniospore was recorded.

RESULTS AND DISCUSSIONS

Morphological characterization of *Hemileia vastatrix*

The urediniospore wall of *Hemileia vastatrix* is hyaline and reniform ($28-36 \times 1-28 \mu\text{m}$), strongly warty on the convex face, smooth on the concave face, and $1 \mu\text{m}$ thick with spherical teliospore. The morphological character of *Hemileia vastatrix* reveals the half-smooth and half-rough surface. Due to a unique combination of three morphological features the genus *Hemileia vastatrix* is distinguished from other genera of rust pathogen which include suprastomatal bouquet sori; urediniospores are

ovoid to reniform with ventral side smooth and echinulated convex dorsal side, and with angular-globose to very irregular teliospores. The helium is distinguished toward the middle of the ventral side in oval urediniospores. Whereas in reniform urediniospores helium is found at the edge of the ventral side. Less perturbation was found in reniform urediniospores compared to oval urediniospores. The gradual increase in the diameter of pale-yellow spots is the first symptom of CLR, before the deposition of orange-colored uredinial on the lower surface of the leaf. On average, mature urediniospores can develop up to 200-300 spines at the dorsal surface, these groups of spines function to grip and hold onto foliar tissues of leaves (Table 2). Beyene et al. (2021) studied the coffee leaf rust and its hyperparasite in dry and wet seasons at 60 sites across southwestern Ethiopia and found more severe leaf rust during the dry season and the wet season in two out of three years seems more hyperparasite. Generally,

coffee leaf rust is more severe at lower altitudes in the dry season and more severe at high altitudes in the wet season.

Worldwide more than 50 physiological races of *Hemileia vastatrix* have been identified (Rodrigues-Junior et al., 1975; Zambolim & Caixeta, 2021). Carvalho et al. (2011) investigate the hidden sexual life cycle disguised within the asexual spore and elucidate new physiological races using computer-assisted DNA image cytometry followed by a modified nuclear stoichiometric staining technique with Feulgen (Figure 3).

CLR occurrence in various elevation

Coffee leaf rust severity was evaluated in three districts, different months at various elevations, from Jan-Dec 2022. Highest disease prevalence was observed in the month of Sep-Oct and Nov-Dec, in Hassan, Kodagu, and Chikmagalur, the lowest was observed during the month of May-Jun (Figure 4A, B).

Table 2. Morphological variation in uredospore of *Hemileia vastatrix* isolates from coffee growing regions in Karnataka, India

Isolates	Spore color	Shape of spores	Echinulation	Elevation	Measurement		
					Length (μm)	Width (μm)	Wall thickness (μm)
CHKM-1	Orange	Reniform	Present	Hunchback	8	4	1
CHKM-2	Orange	Reniform	Present	Convex	6	6	1.2
CHKM-3	Orange	Reniform	Present	Convex	10	2	2
CHKM-4	Yellow	Oval	Present	Convex	6	4	1.5
CHKM-5	Yellow	Reniform	Present	Convex	4	6	2.5
CHKM-6	Yellow	Oval	Present	Convex	4	8	1.2
CHKM-7	Orange	Reniform	Present	Convex	10	4	1.5
CHKM-8	Yellow	Reniform	Present	Convex	8	2	1.6
CHKM-9	Yellow	Reniform	Present	Convex	7	3	1.6
HSN-1	Yellow	Oval	Present	Convex	8	3	1.5
HSN-2	Yellow	Reniform	Present	Hunchback	6	5	2.5
HSN-3	Orange	Oval	Present	Hunchback	7	2	1.1
HSN-4	Yellow	Reniform	Present	Hunchback	5	2	1.8
HSN-5	Yellow	Reniform	Present	Hunchback	3	3	1.5
KDG-1	Orange	Reniform	Present	Hunchback	6	2	1.6
KDG-2	Yellow	Oval	Present	Hunchback	8	3	1.3
KDG-3	Orange	Reniform	Present	Hunchback	4	2	1.4
KDG-4	Orange	Reniform	Present	Hunchback	6	3	1.3
KDG-5	Orange	Reniform	Present	Hunchback	3	1	1.0
KDG-6	Orange	Reniform	Present	Hunchback	5	3	1.4
KDG-7	Orange	Reniform	Present	Hunchback	5	3	1.8
KDG-8	Orange	Reniform	Present	Hunchback	7	4	1.3
KDG-9	Yellow	Oval	Present	Hunchback	9	4	1.6
KDG-10	Yellow	Reniform	Present	Hunchback	6	4	1.2
KDG-11	Orange	Reniform	Present	Hunchback	5	1	1.0
KDG-12	Yellow	Reniform	Present	Hunchback	7	3	1.5
KDG-13	Yellow	Oval	Present	Hunchback	8	4	1.3
KDG-14	Orange	Reniform	Present	Convex	4	2	1.2
KDG-15	Yellow	Reniform	Present	Convex	8	3	1.0

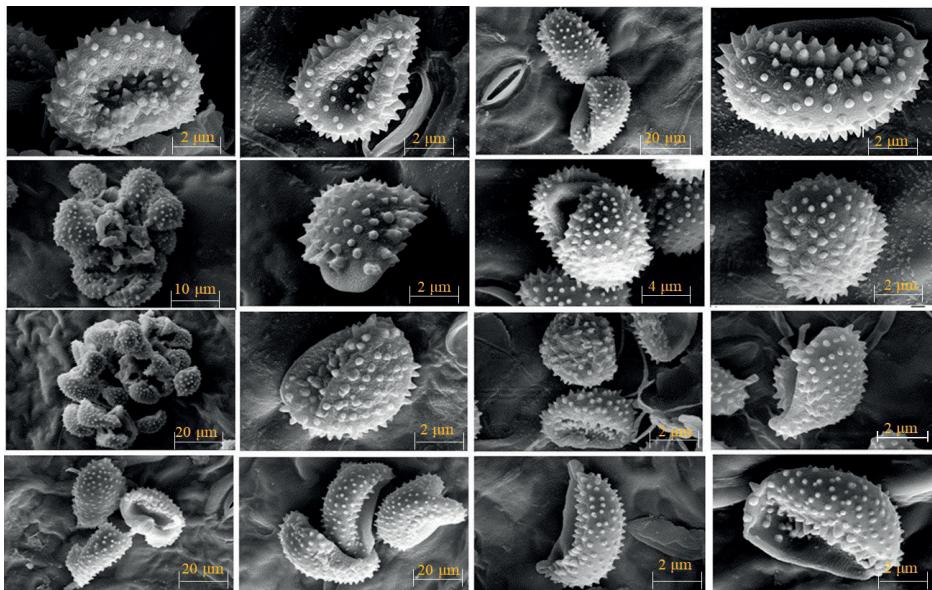


Figure 3. Morphological variation of *Hemileia vastatrix* under scanning electron microscope

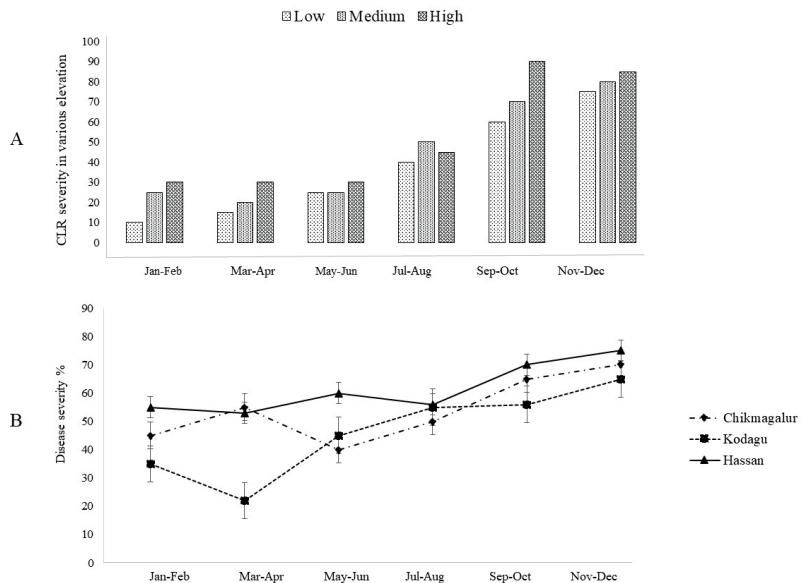


Figure 4. CLR incidence in various elevation (A) and disease severity index in Chikmagalur, Kodagu, Hassan during different seasons during Jan-Dec 2022 in Karnataka, India (B)

Variation in the size of urediniospores

Hemileia vastatrix spore size varies in length, width and wall thickness. Color of the spore varies from yellow to orange, shape of spores varies from reniform to oval. The presence of echinulation was observed on all spores. Elevation varies from hunchback to convex

thirty urediniospores (30×60 leaves = 1,800 spores) were measured per leaf under a light microscope ($\times 100$) (CX23 Olympus). Through the epidermal cells, basidiospores germinate directly and it is related to the monokaryotic phase. The dikaryotic phase initiates with the formation of urediniospores germ tube and

elongation for surface recognition. After the germination of uredinospores germ tube on subsidiary cells, results in the formation of appressorium over the stomata. The dikaryotic phase is a crucial stage for rust fungi for the development of CLR when in contact with the lower side of the leaf. The hydrophobic nature of uredinospores and the cuticle wax layer in the host leaves enhances the adhesion of uredinospores.

The presence of the spines on the uredinospores provides the mechanical grip for adhesion (Lorrain et al., 2019; Voegele et al., 2009; Mapuranga et al., 2022; Panigatti et al. 2025).

The diagrammatic scale is a tool to determine the close to the real value of CLR on *arabica* coffee leaves. The instigation of *Hemileia vastatrix* infection on coffee leaves, like other rust fungi, comprises specific events, including adhesion to the host surface, germination of urediniospore, formation of appressorium over stomata, penetration and inter- and intracellular colonization (Braun & Howard, 1994).

Aristizábal (2024) studied the CLR incidence in three coffee farms from the South Kona district of Hawaii Island. The study focuses on the early detection of CLR incidence, management practices, agronomic information, and evaluation of the cost of controlling CLR. The effective measure to control CLR sprays of fungicides with good coverage.

CONCLUSIONS

The major threat to sustainable and profitable coffee production is coffee leaf rust disease all over the world. CLR continues to be a major task to control for coffee growers in Karnataka. In the present study, the severity of the CLR epidemic was studied in 29 different localities in different months. The incidence of CLR is highly variable at various altitudes. The result showed the various morphological variation of *Hemileia vastatrix* was observed under a microscope and also through a scanning electron microscope. Even though there are several approaches to control, it is necessary to address integrated approaches to manage the disease using improved biocontrol agents through the implementation of new tools and techniques.

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