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Genetic diversity of *Hemileia vastatrix* in coffee plantations

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled "Genetic diversity of *Hemileia vastatrix* in coffee plantations" independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In 15.04.2023

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Ekaterina Riazanova

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Abstract

The presented bachelor's thesis is devoted to the current state of knowledge about the genetic diversity of *Hemileia vastatrix* on coffee plantations, which is one of the most dangerous diseases of the coffee world that can affect the entire plantation and damage most of the coffee trees on it. Literature studies have provided information on species: taxonomy and history, geographic distribution, ecology, life history and disease control, molecular pathogenicity and economic importance and analysis of the genetic diversity of *H. vastatrix*, which influence the understanding of the general principle of distribution and control of this disease. The aim of the study was to assess the relationship between the genetic diversity of *Hemileia vastatrix* and its impact on coffee cultivation, and to identify gaps in knowledge about genetic diversity. Despite its destructiveness, worldwide distribution and economic impact on the production of such an important cash crop as coffee, *H. vastatrix* is one of the most serious diseases of the coffee tree.

Key words: disease, *Hemileia vastatrix*, DNA markers, genetic diversity

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1. Introduction

Hemileia vastatrix is a biotrophic fungus that causes a devastating disease called coffee leaf rust (CRL), which has now spread throughout the world where coffee trees are grown. Coffee rust disease is one of the most common diseases that causes outbreaks in coffee plantations, most often the pathogen attacks Arabica coffee, causing significant economic damage and serious consequences for coffee (Silva et al. 2022).

The first official confirmation of the appearance of the disease occurred in 1861 in East Africa, later this pathogen was described as *Hemileia vastatrix*, and after another 8 years, the disease reached Ceylon (now Sri Lanka) with the help of the spread of spores, where it led to a powerful blow to the destruction of coffee plantations (Talhinhas et al. 2017).

One of the notable signs of the disease is yellow or orange spots on the leaves, which enlarge over time and form spherical clusters of urediospores, which perform an infective function. When a tree becomes infected with coffee rust, the infected leaves fall off, leaving the trunk of the tree bare of leaves. There are several ways to combat the disease, and one of them is a chemical method of control, with this method, the plant is treated with fungicides, the second method is the genetic method of control, its principle is to use more resistant varieties (De Melo et al. 2019).

A more effective method was considered the method of genetic control, but in the last decade the effectiveness of this method has decreased significantly due to the development of new races of the pathogen. In order to provide measures to control a pathogen, it is necessary to know the genetic diversity of the species. To provide control measures for the pathogen, it is necessary to know the genetic diversity of the species, which gives hope for the creation of new species that are more resistant to coffee rust (Bekele et al., 2022).

2. Aims of the Thesis

The main objective of this thesis was to determine why it is so difficult to control the highly devastating *Hemileia vastatrix* disease in coffee plantations, the specific objectives are as follows:

1) To review the present levels of knowledge on the genetic diversity of *H. vastatrix* and its influence on the appearance and spread of rust on coffee leaves

2) To identify gaps in knowledge and research related to the spread of coffee rust

3. Methodology

The work is based on the analyses of information obtained from selected literature using knowledge from research articles, books and scientific sources such as Scopus, Encyclopedia Britannica and Web of Science, through which information was searched related to the current state of knowledge about the genetic diversity of *Hemileia vastatrix* in coffee plantations, which is one of the most dangerous coffee diseases that can affect the entire plantation and damage most of the coffee plants. Despite its destructive power, worldwide distribution and economic impact on a vital cash crop like coffee, there are some knowledge gaps in our understanding of this disease. Literature studies will provide basic information about the type of coffee rust in coffee production areas, which influence the understanding of the general principle of the distribution and control of this disease in coffee plantations. The second part of the work is devoted to the genetic diversity of the pathogen, which discusses the principles of detecting coffee rust using genetic markers.

4. Literature Review

4.1. General introduction on coffee

Coffee (*Coffea* genus) is a woody evergreen plant that is currently distributed mainly in tropical or subtropical areas in more than 70 countries of the world. One of the favorable growing areas is close to the equator, and coffee is common in countriesfrom South America: Brazil and Colombia, from Central America: Guatemala and Honduras, from Africa: Tanzania and Ethiopia, or from Asia: India, Indonesia and Viet Nam (FAO 2019) (Figure 1).

Table 1. Top 10 largest coffee producing countries in 2021 (FAO 2019).

Country	Production (thousand t)	Area harvested (thousand ha)	Yield (t/ha)
Brazil	2993.7	1836.7	16.2
Viet Nam	1845.0	653.1	28.2
Indonesia	765.4	1249.6	6.1
Colombia	560.3	840.1	6.6
Ethiopia	456.0	685.2	6.6
Honduras	400.6	336.3	11.9
Uganda	374.7	692.5	5.4
Peru	365.5	454.7	8.0
India	334.0	422.9	7.8
Guatemala	226.7	363.8	6.2

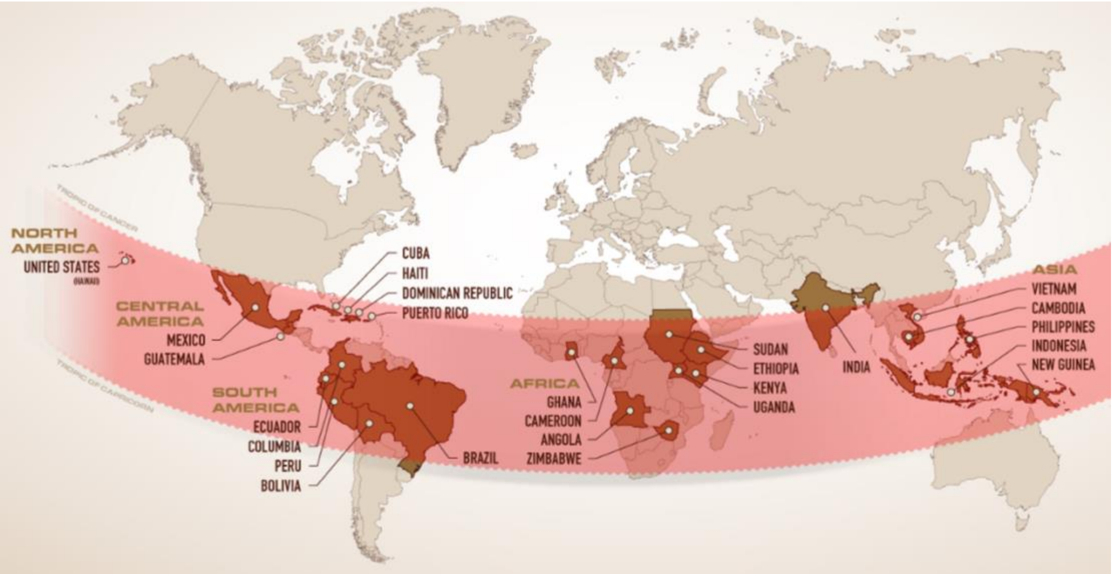

Figure 1. Map of coffee producing countries in the world (Source: Deshmukh et al. 2021).

The coffee plant prefers to occupy the lower tiers in the forest, where it can grow to various heights, from small shrubs to trees that can reach over 10 m in height, although the average height reaches 4.5-6 m. It is worth emphasizing that the color of coffee leaves can be different and therefore there are colors from yellowish green to dark green, there are also elliptical shapes that are opposite on the branches and slightly wavy along the edges and often have noticeable venation. The plant has small white-pink flowers that usually last for a few days before the flowers drop off, the process of pollination takes place, where fruits are then formed, from which coffee drinks will be made in the future. The fruit itself, known as the "coffee cherry", is a drupe with one or two seeds and can be of different colors, such as red, yellow, orange or black, depending on the degree of maturity and type of plant (Farah & Dos Santos 2015).

The genus *Coffea* belongs to the Rubiaceae family, which includes more than 6500 species (Lokker et al. 2014). The most commonly cultivated species are Robusta coffee (*Coffea canephora*) and Arabica coffee (*Coffea arabica*). *Coffea canephora* is a coffee with a higher yield but lower palatability than *C. arabica*. Generally, *C. canephora* is divided into many varieties, with a few examples being:

- Robusta
- Conylon
- Kouilou

It is worth noting that Robusta coffees are also hardy against various diseases such as coffee rust (*Hemileia vastatrix*) or coffee berry diseases (*Colletotrichum kahawae*). On the other hand, *Coffea arabica* has its own characteristics, that are representative of this species, such are susceptibility to diseases, but nevertheless the coffee tastes quite pleasant with low caffeine. *Coffea arabica* also has quite a few varieties such as:

- Typica
- Bourbon
- Mocha

In addition to the fact that coffee is one of the most popular drinks, it can also be used in cooking, as a flavoring additive for mousses, jelly, pastry cream or ice cream. Its use can also be seen in cosmetic areas, such as in the form of coffee grounds for the preparation of scrubs, masks, peels, creams (Pohlan & Janssens 2010). Because coffee-based cosmetics help to quickly restore the skin and put it in order, improve its elasticity, get rid of dryness and flaking. Coffee beans have regenerative properties, as they are rich in antioxidants. These substances help to rejuvenate the skin and make it fresher. Coffee is also found in the pharmaceutical industry, where it is used to help reduce the risk of certain diseases (Farah & Dos Santos 2015).

4.1.1. Economic relevance of coffee

Coffee is one of the most popular tropical evergreens and one of the best-selling agricultural commodities in the world, grown in over 70 tropical countries around the world. However, with the advent of such a devastating disease as coffee rust, coffee production has become unprofitable and costly. The world's coffee genetic resources are also depleted every year due to deforestation and excessively low coffee prices, which is why farmers simply refuse to grow coffee trees in favor of other crops that would bring more profit (Villarreyna et al. 2020). The biological aspect that also affects coffee production is climate change, which in turn brings a variety of diseases and pests, and the same fluctuations in drought or constant rainfall, all of these listed points expose the existence of coffee-growing countries to difficulties. But given the past years, the coffee economy has changed its strategy, in the sense that the market price has decreased and resources have increased, but the interest in coffee in the world has thereby increased (Pham et al. 2019). In order to make coffee production more sustainable, it is not the quality of coffee that should be improved through the use of ecological coffee growing methods. For example better water management, because when coffee is grown on coffee plantations, chemical effluents are released into rivers, which negatively affects the environment, although farmers just need to use less pesticides. Furthermore, water management is not the only issue, the crop management is also important, since, for example, the same coffee husks can be used as fuel. (Ecotactbags et al. 2023). It is also worth to improve the lot of small farmers through trade and, of course, providing or offering assistance to producers (Linton et al. 2008). International coffee organizations usually offer this assistance because they support coffee farmers, their goal is to develop the coffee sector and reduce poverty in developing countries that grow coffee (European Coffee Rederation et al. 2020). After all, if economic conditions do not improve, then coffee will be vulnerable to outbreaks of various diseases on coffee plantations, devastating diseases such as *Hemileia vastatrix* (Villarreyna et al. 2020).

4.2. Taxonomy and morphology of *H. vastatrix*

Domain: Eukaryota

Kingdom: Fungi

Phylum: Basidiomycota

Class: Urediniomycetes (= Pucciniomycetes)

Order: Uredinales (= Pucciniales)

Family: Pucciniaceae

Genus: Hemileia

Specific epithet: vastatrix

There are many diseases associated with fungal infection of coffee trees around the world, but one of the most common diseases is coffee leaf rust (CLR), caused by the biotrophic rust fungus *Hemileia vastatrix*. This rust belongs to the genus *Hemileia* and the phylum Basidiomycota, as described by Berkeley and Broome in 1869. This genus includes 42 species that are found in the tropical or subtropical regions. In recent years, with the help of molecular biological studies, some aspects of the taxonomy of this order have been clarified, as a result of which it has been placed in the class Pucciniomycetes (=Urediniomycetes). This parasitic fungus extracts nutritious products only from the cells of a living organism, and coffee plays the role of the host in this case. It is known that this fungus has dikaryotic uredospores that are directly related to the infection of the plant (Figure 2), and this representative of *H. vastatrix* the uredospores are reniform, having these parameters 28–36 × 18–28 μm, and the uredospores also have a hyaline wall, usually warty on the convex side with smooth ventral side and having a thickness of 1 μm (Talhinhas et al. 2017).

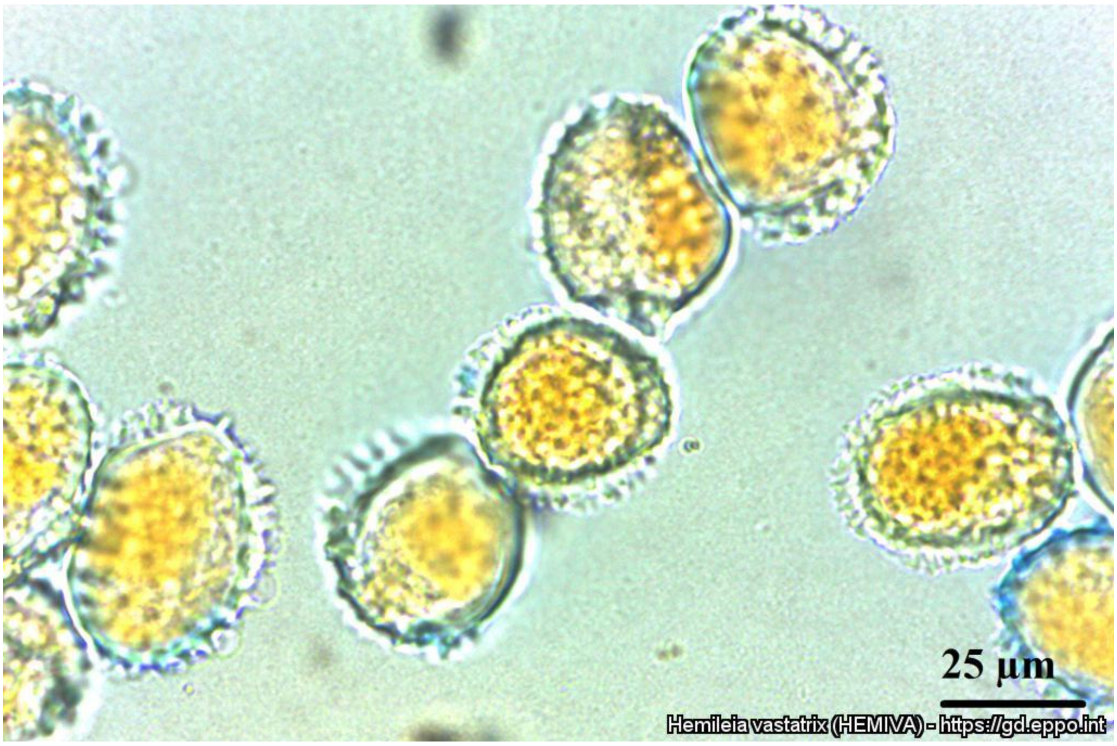

Figure 2. Uredospores of coffee leaf rust, collected in Chiapas, México (Source: Alvarez et al. 2023).

The biotrophic fungus also produces sexual spores or basidiospores, which do not take an indirect part in infecting the coffee leaf, like urediospores, which work as one of the dominant factors in infecting the plant. In urediospores or asexual spores, the processes of the emergence of the process of nuclear fusion (karyogamy) were noted, and then the spores go through the stages of meiosis. In these processes, sexual reproduction, which is hidden under the guise of asexual spores, is also called the process of cryptosexuality, which reduces the resistance of the plant. Cryptosexuality explains the rapid and frequent spread of the biotrophic fungus. This phenomenon plays an important role in studying the processes of coffee selection and the emergence of new races in *Hemileia vastatrix*. (Carvalho et al. 2011).

4.2.1. Cycles and spread in the environment

Hemileia vastatrix is a parasitic or biotrophic fungus, which means that such a fungal pathogen feeds on living plant cells. The first stage in the life cycle is the adherence of urediospores to the lower surface of the leaf, where it begins its germination. (Figure 3). At this stage, the first signs of infection begin to appear as small and yellowish spots with granular dust on the underside of the leaf, the spots increase in size over time, and the dust forms new infected pores. And if at the same time favorable and comfortable conditions for the disease are provided, then the infectious process will be much shorter (Silva et al. 2022).

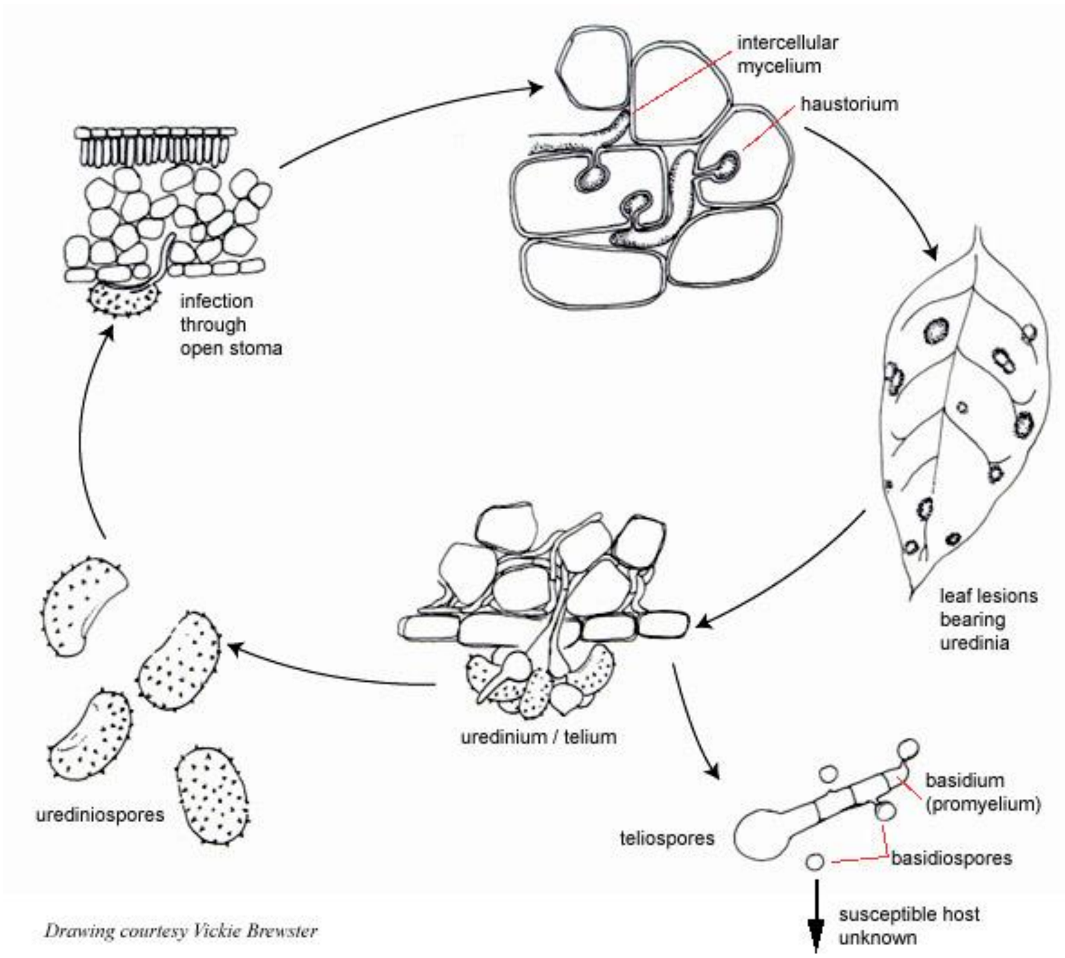

Figure 3. Coffee leaf rust life cycle (Source: Arneson et al. 2011).

Optimal conditions for development of *H. vastatrix* are represented in the form of increased humidity, e.g. due to constant heavy rains, temperature for spore germination within a range of 21-25 °C and lack of direct sunlight. When urediospores hit the leaf, the process of introducing hyphae to infect the coffee leaf begins, which lasts from 5.3 to 8.5 hours, but when drought sets in, the infection process stops, since the dry climate directly affects the development of the coffee leaf. After the hyphae have penetrated on the coffee leaf, the disease also begins to penetrate through the stomata into the leaf, where the process of parasitism occurs, when the structures of the fungus begins to extract nutrients for its growth. The subsequent spread of the pathogen manifests itself in the form of a continuous penetration of a huge number of hyphae and haustoria, which also represent the organ of nutrition in parasitic plants. When the hyphae penetrate into the substomatal cavities at this moment, the hyphae begin to dissect into separate heterogeneous elements that form protosoria and within a few weeks urediniospores appear in the form of large accumulations (Silva et al. 2022).

4.2.2. Infection steps and reproduction

As already mentioned, as soon as the accumulation of urediospores occurs, they begin to germinate in the leaf tissue, thereby causing colonization, because this pathogen uses adhesins to use the relationship with host cells for subsequent infection. This step is very important in the stage of infection, because at this stage the process of differentiation of specialized cells of the fungal pathogen takes place, which are used to infect the cells of the plant host (van Belkum et al. 2021).

After the suppression has formed, the fungal pathogen begins to penetrate through the stomata in the host cell through the hypha, which then grows into the substoma. It is worth noting a remarkable feature of the *H. vastatrix* pathogen that the penetrating hyphae also form two new branches, which, when observed under a microscope, resemble the shape of an anchor (Talhinhas et al. 2017) (Figure 4).

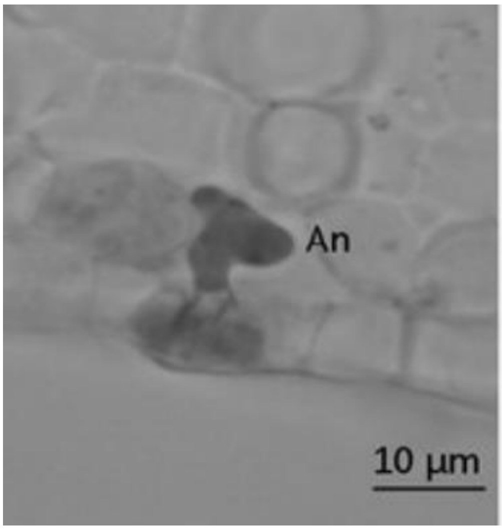

Figure 4. Germinated urediospores (Source: Diniz et al. 2012).

Where each side branch gives rise to the formation of haustoria, which infect the stomatal daughter cells. Thus, the pathogen continues to spread, forming a huge number of intercellular hyphae and haustoria. Three weeks after infection, urediniosporium sori begin to pass through the stomata in a bouquet. At this stage, chloroses are already beginning to be seen, which protrude through orange-colored pustules, which are identified as one of the main signs of coffee rust infection (Talhinhas et al. 2017).

4.2.3. Origin and history of coffee rust disease

The pathogen was originally discovered in 1861 in East Africa by an English explorer, but one of the first and major outbreaks of the disease occurred in 1869 in Ceylon (now renamed Sri Lanka). That same year, scientists at Berkeley and Broome described an unknown coffee-destroying disease as a biotrophic fungus now known by the Latin name *H. vastatrix*, or coffee rust (Talhinhas et al. 2017).

Within 100 years, the coffee rust disease had spread to almost every region of the world where coffee was grown. At first, many farmers in the early 90s the 20th century were of the opinion that the disease was under control, but their opinion changed dramatically when, in the 2000s, a phenomenon called the "Big Rust" broke out across America, which spread with great speed in countries like Mexico, Peru and the Dominican Republic. In subsequent years, due to the spread of the disease, coffee production began to bring huge losses to those countries that were growing coffee, in the same Colombia, the profit from coffee production decreased by 30%, which seriously affected the country's economy. The disease did not stand still and in 2013 it spread from South America to Central America and Mexico, where the disease also caused damage, for example, in Honduras, coffee production decreased by as much as 25%, in El Salvador, coffee rust, the damage caused reached almost 50%, and in Mexico about 20%. (McCook & Vandermeer 2015). Currently, the attack of the biotrophic fungus has shifted more to the countries of South America, where countries such as Peru and Ecuador have been attacked due to favorable climatic conditions. Although coffee rust has an excellent ability to adapt to different climatic conditions, it still prefers areas where the weather is warmer and more humid. Because in such countries there is a high probability of causing a secondary blow to the affected plants from coffee rust, which affects the production of coffee next year. The spillover effect not only reduces coffee production, but also has a huge impact on farmers, who also go into the red because it brings the price of coffee down and, after that, farmers begin to receive less income for the crop that was exported. And, of course, the paradox is that the profits from coffee production were as low as possible, and the prices for fertilizers and fungicides that fought the disease were high, which put farmers at a disadvantage when their costs exceed the profits (McCook 2006). Such unpreparedness of people and suitable climatic conditions made it a favorable environment for the spread of the disease, but the fungus did not cause such devastating consequences as, for example, in Ceylon when it first appeared. Still, in America, farmers were more prepared for such problems, because there were scientific institutes of coffee, which, among other things, were engaged in the study of various diseases, including coffee rust. In 1990s the institutes welcomed the use of chemical control agents or the breeding of varieties resistant to the disease, although research showed that fungicides were ineffective, and plant breeding provided new ideas for controlling the disease. In 1989, no international agreement was signed on coffee that would limit production and keep prices profitable. This was the result of the removal of many restrictions on the volume of coffee production and price regulation, because the heads of state could not agree on new quotas. In this regard, coffee production increased by almost 40%, which returned countries to repeated failures and losses, because producers worked at a loss, and farmers received only a small part of the production. The Big Rust situation showed that even small changes in weather patterns and instability in the global economy that manifest themselves in the form of imbalances and the appearance of diseases, but also helped to identify problems in the future in coffee production, because the ability to learn from mistakes will help to foresee the future. consequences (McCook & Vandermeer 2015).

4.2.4. Disease symptoms and signs

It is worth noting that infection with the fungus *Hemileia vastatrix* occurs on the leaves of the coffee tree. And one of the first symptoms that can be seen with the naked eye is small light yellowish spots on the top of the leaf (Figure 5). Over time, the newly formed spots expand until they form orange-yellow spots, but already on the underside of the leaf, which appear as brown spores (urediniospores), which indicates that the plant has undergone an infection by the fungus. Also, infection with the fungus can occur anywhere on the leaf of the plant, but basically the infection chooses places around the edges of the leaves, because there is a retention of water droplets due to rain or morning dew (De Melo et al. 2019).

But unlike other leaf rusts, the coffee rust fungus does not damage the epidermis of the leaf, but forms spores that penetrate through the stomata of the plant, which means that the fungus forms a pustule, which eventually begins to turn black, causing the leaves to fall, and subsequently exposes the whole tree (Britannica 2017). As a result, the trees not only fail to bring a fruitful coffee crop, but also die within a few years (Yirga et al. 2020).

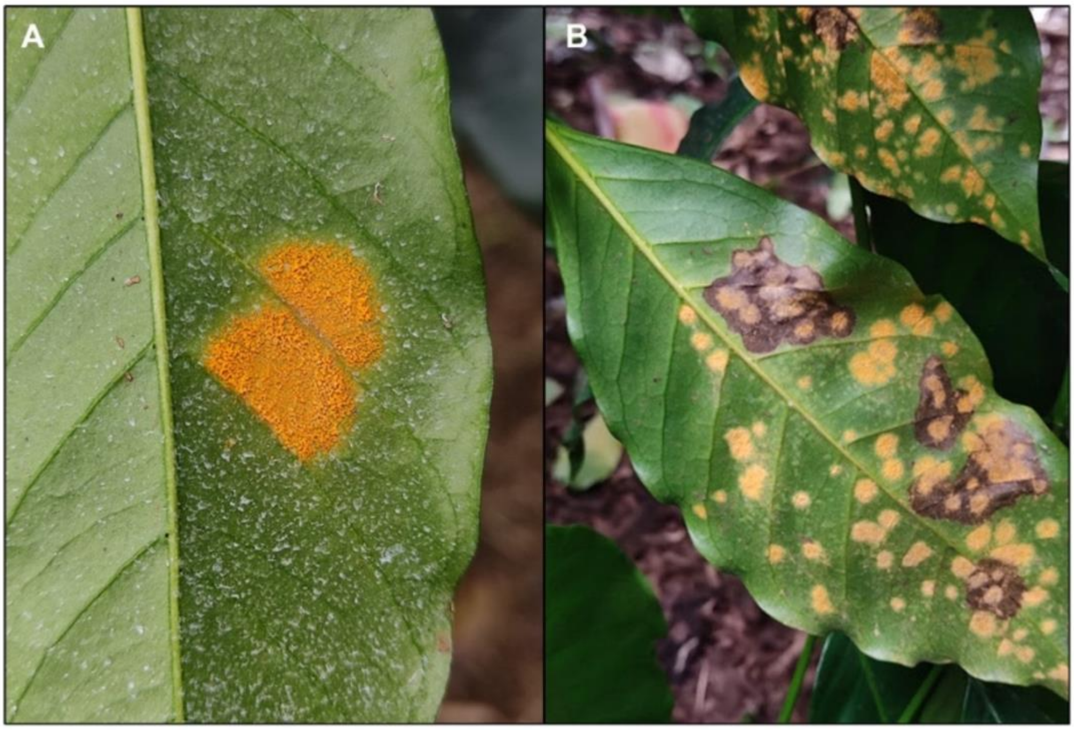

Figure 5. Rust on coffee leaves (Source: Aristizábal & Johnson 2022).

4.2.5. Economic significance of the pathogen

Coffee rust infestation on the plant causes an almost 40% yield loss depending on weather conditions, resulting in economic losses. Currently, *C. canephora* and *C. arabica* are the most commonly used, accounting for almost 60% of coffee production (Rodrigues et al. 2022). After all, the disease initially affects the growth and fruiting of coffee, causing losses exceeding two billion USD every year. But not all countries have suffered similar damage, if we take Hawaii as an example, where this disease was not observed until October 2020, because Hawaii benefits from its geographical isolation from other countries, which accordingly gives an advantage. Also in Hawaii, strict rules were observed regarding the import of new plants, but unfortunately in the winter of 2020 *Hemileia vastatrix* spores were found and therefore, closer to summer, the disease had already swept the whole island. Inflicting significant losses on coffee production, because it is very difficult to eradicate invasive species (Ramírez-Camejo et al. 2022).

However, in many other countries, coffee rust has dealt a serious blow to coffee production, for example in Kenya, the economic loss is due to the fact that Kenya has several rainy seasons, which are good weather conditions for the reproduction of biotrophic fungus. Understanding the cause of the disease allows us to control the disease (Gichuru et al. 2021)

4.3. Methods of control of *Hemileia vastatrix*

The pathogen can be controlled in two ways, genetic control or chemical control, which will reduce the harm caused by the disease. Consider the genetic control that originates in Central America, where they were based on coffee production from old coffees such as Typica and Bourbon, but there were also cross varieties such as Villa Sarchi or Caturra. Such crossed species have a high ability to bear fruit on the lower branches of the plant, but, unfortunately, are most often susceptible to coffee rust disease. Therefore, in 1989, the offspring of the Timorese hybrid was bred, which began to be used not only in the countries of Central and South America, but also in the countries of Asia and Africa in breeding programs, since these hybrids became the starting point for breeding new varieties resistant to the disease. And so in the 1980s, mass crossbreeding began to produce a satisfactory generation, and so the Katimor variety appeared in the world, which gave the first hopes, because it had a high resistance to coffee rust disease. The genetic crossbreeding of hybrids did not stop there, and so a new genetic improvement project began in the 1990s. therefore, 20 hybrids from the F1 generation were taken, of which two F1 hybrids showed the best results during the experiments: Centroamericano and Millennium, as they had a high resistance to coffee rust disease and higher productivity than Catimor and Caturra varieties. Taking into account all the advantages that the hybrids showed during the tests, it is worth noting that the quality of coffee cups of the presented varieties is not very good. And unfortunately, there is currently no variety that can have good quality coffee cups and also be resistant to all existing coffee-related diseases, but as a strategy, a farmer can combine, for example, two genetic varieties that are resistant to various diseases to reduce the risk of diseases. Meanwhile, the most common way to prevent the spread of coffee rust is to use a chemical method to combat coffee rust disease, this was one of the first ways to deal with this disease. Since the onset of the disease, farmers have been using fungicides to kill pathogenic fungi. But before applying fungicides, it should be borne in mind that CRL is most actively manifested in the fruiting phase of the plant, and therefore the time before the start of the rainy season is this period that is considered one of the ideal options for the use of chemicals. Some variants of fungicides were presented, as one of the first presented was a sulfur-based fungicide, but unfortunately its effectiveness with the defeat of the disease was low. Further, a copper-based fungicide was used and the results of the use were good, because the principle of this fungicide was to block the breathing of the spore of the fungus, thereby hitting the biomembrane of the fungus. It is this fungicide that is best used if the area affected by the fungus is less than 10%, but if the percentage of damage is greater, then it is already better to use therapeutic fungicides, because their operation mode is to get into the plant tissue so that in the event of an attack by the fungus, the plant could defend itself. Also, during the research, a group of fungicides was presented - triazoles, which suppress ergosterol in fungi, from which coffee rust develops, because the fungus cannot receive enough enzymes for development (De Melo et al. 2019). Both genetic and chemical resist the disease, thereby reducing the spread of the fungus (Pereira et al. 2021).

4.3.1. Prevention of coffee leaf rust

There are several solutions to prevent the development of the coffee rust pathogen *Hemileia vastatrix.* But first, it is worth paying attention to the fact that for a fertile crop, it is necessary to take appropriate measures to protect the leaves of the coffee plant in order to reduce the risk of developing a biotrophic fungus. After all, the role of the leaves plays a huge role, firstly, in the metabolism of the plant, because this process supports life, helping the plant grow and multiply and providing the plant with nutrients, and secondly, healthy leaves give a greater variety of fertile fruit crops and thus a large harvest. One of the measures that can help prevent the development of the epidemic is coffee pruning, in which weakened, diseased or old branches are removed to support the growth and fruiting of plants, because the quality and quantity of the future crop depends on pruning. The presented measure is best carried out when the first signs of exhaustion begin to appear, because until this moment it is almost always difficult to predict when the plant needs pruning, because it all depends on the care of the coffee tree and, accordingly, the care of the coffee tree is different for each farmer.

Plant pruning is divided into several types: one of these types is selective pruning, the essence of which is to observe the plant and selectively remove damaged leaves.

Another way is cropping by rows, where entire rows are removed when coffee rust appears to increase the chance of keeping other rows where coffee rust has not been detected. And the third method is cropping, which means removing all the plants that have grown in the field, where the farmer himself chooses the size of the plot to remove the plants. When a coffee plant is removed from a certain area, the presented area is advantageous in that the farmer can grow different agricultural products on the empty area than if the area was empty, because it would bring a large cost of maintaining the area. At the onset of the rainy season, buds begin to sprout on an already pruned coffee tree, giving new shoots, which must be removed to ensure good ventilation and proper formation and development of the plant, while leaving only strong shoots in the axils of the leaves. Also, pruning and removing shoots improves sunlight penetration and reduces moisture, which is an unfavorable condition for the spread and appearance of coffee rust (De Melo et al. 2019).

4.3.2. Ecology

Infection with the fungal disease *H. vastatrix* begins with the formation of urediniospores, which, with the subsequent increase in infection, cause more and more new uredinospores. For the disease has an uninterrupted character due to the fact that coffee is an evergreen plant and therefore their foliage persists throughout the year. Water is one of the key points for the germination of urediospores, because sufficient water is needed for development, which can manifest itself for example in the form of rain, because the rainy season is the ideal time for coffee infestation. However, under unfavorable conditions such as dry seasons, the *Hemileia vastatrix* inoculum will reside in plant tissue, which will be seen as mycelia that survive for about a few weeks. When the conditions for fungus infection are met with enough water and climatic conditions will also be taken into account, because the temperature will begin to have a great influence on the development of uredospores, because CRL prefers warmer climatic conditions for the development of the pathogen, which take their range from 15-30 °C. At lower temperatures, the infection process slows down. It is worth noting that the distribution of uredinospores to the plant occurs in several ways, such as rain or wind, or animals and humans (Arneson al et. 2015).

4.4. Genetic diversity

Every organism on Earth has a unique hereditary code, represented as DNA, organized into genes that determine the functions of the organism. Genetic diversity refers to the range of genetic variation within and among individuals, populations, and species. It includes the variety of alleles, gene combinations, and genetic traits that are present in a given population. The importance of genetic diversity lies in the fact that the species adapts better to changing external conditions, because the survival of the species directly depends on this (Minter et al. 2021). But also genetic diversity determines genetic variability, which, in turn, is the cause of the diversity of living organisms on earth. However, the importance of genetic diversity is evident at both the individual and population levels (Mukhopadhyay & Bhattacharjee 2016). An important role is also played by the fact that with an increase in genetic diversity, species have the opportunity for the subsequent formation of their own kind, which is a plus for the spread and development of the pathogen (Rauf et al. 2010).

4.4.1. General classification of genetic diversity

To assess the analysis of genetic diversity, methods are usually used that involve the use of molecular markers or genetic markers, both of the presented markers are based on DNA. As soon as there was a limitation of genetic markers that had a phenotypic basis, molecular markers that were stable enough for analysis began to appear in the world (Mondini et al. 2009). Also, markers are divided into markers that have PCR reactions in their analysis and those that do not have PCR reactions (Rauf et al. 2010).

Molecular methods to assess the genetic diversity of the fungus *Hemileia vastatrix*, for example using the following DNA markers (Bekele et al. 2022):

- Simple sequence repeats (SSR) = microsatellite analysis

The simple sequence repeats method, which is represented by a simpler name as microsatellites, this method is most often used in the practice of molecular markers, because SSR markers have a high level of biodiversity presented in the form of polymorphism, which allows you to show more effective and informative results. The principle of operation of the SSR method lies in simple repetitive sequences, which are sometimes subjected to errors in DNA and thereby cause mutations, due to which polymorphism appears (Mason et al. 2015).

- Random amplifield polymorphic DNA (RAPD)

The RAPD method is a modification of the PCR method and involves the use of non-specific primers and obtaining DNA products of amplification of individual sections of template DNA. This method is used in population genetics or in plant breeding, where the necessary costs for analysis do not require large costs and efforts (Kumar & Gurusubramanian 2011).

- Amplified Fragment Length Polymorphism (AFLP)

The presented method of amplified fragment length polymorphism is a rather complex method of analysis, consisting of several stages. One of the exclusive features of AFLP is the ability to scan the entire genome for traces of polymorphism, which allows the use of DNA of any complexity and origin (Blears et al. 1998).For example, in a sugar cane study, this method provided more knowledge about the ancestry of the branch, which added more biodiversity awareness (Rauf et al. 2010).

4.4.2. Genetic diversity of *Hemileia vastatrix*

Coffee is critical to the economies of tropical countries that export this product. But in the last decade, the coffee industry was on the verge of change when devastating diseases appeared in the world that hit the coffee sector, and one of these diseases is the coffee rust disease caused by the biotrophic fungus *Hemileia vastatrix*, which caused huge losses in producing countries. After all, one of the main problems is the progressive and rapid spread of the pathogen, usually the disease is carried by spores downwind, but the sources can also be carried by animals or even humans (Bekele et al. 2022).

There were various methods to control and combat the disease, such as the use of fungicides or the use of *Hibrido de Timor* hydride as a resistant variety to the disease. However, more and more varieties became unable to resist the infection and pathogen development. After all, a significant increase in the genetic variability of *H. vastatrix* led to the emergence of new races of the pathogen, which are well adapted to different environmental conditions. The significantly rapid emergence of new races in the pathogen is explained by its cryptosexuality (Cabral et al. 2016). When latent meiosis and sexual reproduction (cryptosexuality) within asexual urediniospores was discovered, it took a step forward in the study and understanding of pathogen behavior, and this response has also been observed in the genus *Maravalia*. As it turned out, the genera *Hemileia* and *Maravalia* belong to the same order of fungi (Pucciniales), which tells us that both genera belonged to one of the old plant lines. It also implies, that the pathogen extends not only to the original primitive genus, but also to those genera with which rust has a common ancestor, which increases the rate of spread of coffee rust (Carvalho et al. 2011).

Approximately 50 coffee rust races have been identified around the world as a problem for disease control, as the Híbrido de Timor hybrid was not resistant to the spread of the pathogen and was attacked. Coffee rust reduces plant immunity and increases the risk of disease because the process of introducing coffee rust genes into host plant structures reduces the activity of coffee plants. Expressive genes have also been found at sites of fungus infection in which genetic information has been converted into protein synthesis when the disease releases proteins to avoid coffee plant immune responses, thereby also suppressing plant immunity (Castro et al. 2022).

However, the history of the emergence and spread of coffee rust (CR) shows us how well this species adapts to changing climatic conditions. Looking at the history of the spread of the disease, the pathogen originated in the island of Sri Lanka and could have spread to South America, where this pathogen was transported by transporting plants that were infected with coffee rust, which proves the good resistance and viability of the spores. According to (Cabral et al. 2016), who conducted a study using AFLP markers, the coffee rust population in Brazil showed a low level of hereditary variability and no clear geographical pattern was found in its distribution. However, genetic variability in Brazil is affected by variability within the coffee rust population (Cabral et al. 2016).

Due to the variability within the population, mutations appear that give rise to the genetic variability of the population that can cause evolutionary changes, which is an advantage for the development of pathogens (Loewe et al.2010). A similar case occurred in Ethiopia, when a group of scientists conducted a study with genetic markers using the SSR simple repetition method showed that the variability is also much higher within populations, which was more than 90%. Coffee rust disease is officially confirmed to have originated in Ceylon (Sri Lanka), but it was also known that the disease appeared much earlier in 1934 in Ethiopia, however the type of race that appeared in Ethiopia did not cause the types of epidemics that were common in other countries of the world and which have also been attacked by coffee rust. In other continents, such as Africa, South America and Asia, studies have also been carried out to determine the level of genetic diversity using DNA markers, which showed a low level of *H. vastatrix* genetic diversity. But an analysis that was carried out in Ethiopia shows that from a genetic point of view, outbreaks of coffee rust disease are due to genetically diverse populations of fungi, whose geographical relationship does not depend on geographical origin. This statement is also confirmed in other rust fungi, which also lacked the relationship between geographic origin and genetic diversity, appearing for example in *Puccinia triticina*, which infects wheat crops. Subsequent studies based on the use of RAPD markers showed a low level of genetic diversity, which is in strong contrast to the studies conducted in Ethiopia using the SSR method, where sufficiently high rates of genetic variability were obtained, which provides knowledge for further research in the field of genetic variety in coffee rust (Bekele et al. 2022). Published research on genetic diversity of *H. vastatrix* is summarized in Table 2.

Table 2. Studies of genetic diversity of *H. vastatrix* in different countries.

Country	Marker used	Level of diversity	Reference
Brazil	AFLP	Low diversity among populations	Cabral et al. 2016
Viet Nam	ITS	Low diversity among populations	Okane et al. 2022
Colombia	SSR	Low diversity among populations	Cristancho et al. 2012
Ethiopia	SSR	High overall diversity	Bekele et al. 2022
Hawai	SSR	Low diversity among populations	Ramírez-Camejo et al. 2022
India	RAPD	High overall diversity	Talhinhas et al. 2017

AFLP: Amplified fragment length polymorphism; ITS: Internal transcribed spacer, SSR: Simple sequence repeat; RAPD: Random amplified polymorphic DNA

5. Conclusions

In this work, I have tried to review all the information about the genetic diversity of coffee rust disease and see what factors influence genetic variability. Scientific studies have shown that the species has a low level of genotypic variability and at the same time an increase in genetic variability, which is partly due to the fact that when the coffee tree is infected, cryptosexuality is present in the life cycle of the pathogen, which allows the rapid spread of spores in coffee plantations, which is one of the main problems in the control of coffee rust disease. It is also worth noting that the main reason for the spread of the disease is the lack of control during the transportation of plants between countries, for this it is recommended to take a more thorough and responsible approach to checking plants before transportation, weather conditions also play a role in the spread of the pathogen, ensuring favorable environmental conditions for the pathogen (humidity and heat) allows the pathogen to spread very quickly and in comfortable conditions, to solve this problem, it is necessary to grow coffee under the crowns of trees, if possible, in order to reduce the ingress of sunlight, thereby reducing the large flow of heat that is necessary for the development of the disease. Tree canopies also stop direct water drops from falling to the ground, helping the soil absorb moisture more evenly without causing excessive moisture. Due to its high viability and rapid spread, control of the coffee rust pathogen is not limited to agrotechnical methods, it requires knowledge of the genetic structure of the fungus. Existing knowledge of the genetic structure of the pathogenic fungus helps to understand how CRL disease can be controlled, but, unfortunately, very little research has been done to date using modern methods, such as sequencing methods. In connection with the circumstances described, further study of the genetic diversity of coffee rust is strongly recommended to create new hybrid offspring resistant to various pathogen mutations in order to protect coffee plantations from the pathogenic fungus *Hemileia vastatrix.*

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