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Review on Resistance Breeding Methods of Coffee Leaf Rust in Ethiopia

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ABSTRACT

Coffee Leaf Rust is one of the most important diseases of Coffee arabica in the world. Even though, Coffee leaf rust was first reported in 1934 in Ethiopia it has never reached to epidemic level to cause eradication of Arabica coffee. This may be as a consequence of long-term coexistence of rust and coffee which created a balanced patho system and high level of horizontal resistance. Currently, coffee leaf rust is widely distributed all over coffee growing regions of the country with varying intensities. Highest diseased trees with mean percent of 42.5 in Kaffa, 41.9 in Illuababor, and 39.6 in Hararghe. The average national infected trees were estimated to 12.9% in 1980 and increased by threefold (36.3%) after ten years in 1990. The first symptoms of coffee leaf rust disease is small discolored spots which develop on the underside of the leaves. These small spots increase in size and are powdered with spores of the pathogen ranging in color from yellowish orange to bright orange. Coffee leaf rust affect plant growth by reducing the amount of leaf area available for photosynthesis, either by occupying leaf area or by inducing defoliation principally of the attacked leaves. There are different resistance breeding approaches; durable resistant methods, Cytological and biochemical resistance mechanisms, Molecular approaches to coffee breeding and other hybridization methods and pure line methods were used today resistant breeding program. In Ethiopia, large genetic diversity of Coffee arabica, high level of horizontal resistance to coffee leaf rust and availability of at least some incomplete resistance might likely protects coffee against rust under prevailing conditions identified coffee plants with partially (incomplete) resistance to coffee leaf rust from lowland forest coffee of southwestern Ethiopia. The presences of such wide range of resistance to coffee leaf rust in wild forest coffee population provide an opportunity to develop and use resistant materials for coffee leaf rust management.

Keywords: *Coffee, Leaf rust, Breeding methods, Ethiopia*

INTRODUCTION

Coffee is a commodity of interest worldwide, especially in over 50 countries involved in its production, trade and consumption. *Coffea arabica* popularly known as Arabica coffee and *Coffea canephora* called as Robusta coffee are the two main species under commercial cultivation contributing 70% and 30% respectively of the total Global coffee production (Anonymous, 2008). Coffea arabica L. (2n = 4x = 44), the only polyploid species in the Coffea genus, is an allotetraploid containing two diploid subgenomes, Ca and Ea, which originated from two different diploid species (2n = 2x = 22), C. canephora and C. eugenioides, respectively (Lashermes et al. 1999). In spite of the low divergence between the two constitutive subgenomes, C. arabica displays a diploid-like meiotic behavior and conforms to disomic inheritance(Lashermes et al. 2000a).

Coffee Leaf Rust (CLR) is one of the most important diseases of C. arabica in the world. It devastated Arabica coffee plantations in Ceylon at the end of the 19th century and was responsible for its replacement with tea plantations. Despite effective fungicides and resistant varieties developed to control rust, yield reductions of 20% or more in various countries arestill caused by the pathogen (Waller, 1982). In Brazil, losses have been estimated to be about 30% and an annual loss of about 4500 tons of coffee was estimated in Kenya in the 1960s. The pathogen prefers a temperature range of 20–28 °C, needs a leaf wetness period only during spore germination and penetrates with the germination hyphae into the stomata of the host. The fungus tolerates longer seasons without rainfall and spores are wind-borne, only attacking leaves and needs no other host for completing the life cycle. Due to the fact that coffee is a perennial host with green leaves all through the year, the pathogen produces only uredinio- and teliospores with basidiospores. Coffee grown in lower altitudes is more predisposed to the disease and suffers more attacks. A heavy infestation of leaves not

only reduces the assimilation area but also results in a complete defoliation diminishing the next year's crop tremendously. More than 75% of the coffee cultivated in the world is susceptible to the majority of physiologic pathogenic races (Guzzo, 2004). Selection pressure against these fungi by the continuous use of fungicides and the wide cultivation of genotypes with lower genetic diversity, results in the constant arising of new fungi pathogenic races (Wagner e Bettencourt, 1965). Because coffee is a woody plant with a long juvenile phase, classical coffee plant breeding is slow and needs implementation of techniques that speed up the selection and phenotipic evaluation of elite genotypes for this resistance (Teixeira-Cabral et al., 2004).

Coffee leaf rust was first reported in Ethiopia in 1934 (Sylvain P. 1958), but the disease had existed for a long time in other countries without causing epidemics or eradications of certain varieties of C. arabica. The long-term coexistence of coffee and rust coupled with the high genetic diversity of coffee populations and a high level of horizontal resistance might have kept the rust at low levels (Van der Graaf 1981). Other factors such as the low average productivity associated with shadeand the existence of biological agents such as the hyperparasite Verticillium lecanii, were also believed to play an important role in maintaining coffee leaf rust at low levels.

Coffee leaf rust caused by *Hemileia vastatrix* is one of the most important diseases of *C. arabica* in the world (Kushalappa and Eskes, 1989). The disease may cause yield losses varying between 10 to 40% (Silva *et al.,* 2006*)* in different countries. In Ethiopia, leaf rust has been considered as minor diseases of coffee since it had never reached epidemic proportion as in other countries. Currently, CLR is widely distributed all over coffee growing regions of the country with varying intensities. The average national infected trees were estimated to about 36.3% in 1990 (Meseret, 1991). Eshetu *et al.* (2000) reported as high as 27% CLR severity in Hararghe region (Eastern Ethiopia). The disease incidence has been increaseing from time to time due to change in coffee production system

The identification proof of the species H. vastatrix by morphological characteristics was assisted by scanning electron microscopic photos of rust sori and urediniospores (Ritschel, 2005). A typical sorus extruding from a stoma on the lower side of the leaves had 15–25 lemon-shaped one-celled urediniospores.

Coffee leaf rust assessments in the rainforests of Ethiopia revealed its presence in all fields differing in incidence with time (season) and location. Generally, rust incurs an estimated yield loss between 10- 40% in different countries (Silva *et al*., 2006) and cost of control with fungicide is very high. In Brazil annual loss was estimated to about 30% (Kusahalappa and Eskes, 1989) and the entailed expense for chemical control add up to equivalent US $100-120/ha (Mutappa*et al*., 1989). Coffee leaf rust was first reported in 1934 in Ethiopia (Sylvain, 1955) but it has never reached to epidemic level to cause eradication of Arabica coffee. This may be as a consequence of long-term coexistence of rust and coffee which created a balanced pathosystem (Eskes, 1989b) and high level of horizontal (race nonspecific) resistance (Van der Graaff, 1981; Meseret *et al.*, 1987; Muller *et al*., 2004). Currently, CLR is widely distributed all over coffee growing regions of the country with varying intensities (Meseret, 1991; 1996). Highest diseased trees with mean percent of 42.5 in Kaffa, 41.9 in Illuababor, and 39.6 in Hararghe. The average national infected trees were estimated to 12.9% in 1980 and increased by threefold (36.3%) after ten years in 1990 (Meseret, 1991). Eshetu *et al.* (2000), IAR (1986) reported as high as 27% coffee leaf rust severity in Hararghe and this might be attributed to the distribution of susceptible host, occurrence of virulent races and the type of coffee production systems. Although the disease has been present for such long period and increasing from time to time, it was considered as minor significance and neither inflicted yield loss nor management strategies have been practiced to combat the disease in the country.

OBJECTIVE OF THIS PAPER IS:

To review resistance breeding methods of coffee leaf rust.

Factors of Proliferation

There are a few factors that must be present for Coffee Leaf Rust to attack and damage coffee plants. These are: a susceptible plant or host; the presence of the pathogen; favorable weather conditions for growth; and agronomic mismanagement or poor agricultural practices. Unsatisfactory practices in plant care and cultivation, along with increased effects of climate change, are thought to be the foremost factors contributing to the strength of the 2013 Coffee Leaf Rust outbreak in the Americas. La Niña's effects, such as increased rainfall, diminished sunlight hours, and more saturated

soil, favors Rust cycles, encouraging an epidemic. Its survival depends on being able to travel between living tissues fairly quickly, and coffee is the only crop it can feed off. Only when trees are improperly managed and thus weakened do they become the perfect medium for the spread of fungus to healthy trees in the area.

Stages of Disease Development

Dissemination

Dissemination occurs through spores that look like yellow or orange powder, found on the underside of the coffee plant's leaves. If conditions permit, the fungus will disseminate its spores among coffee trees in the same plot, causing many trees to go through the same stages at the same time. Additionally, the fungus will spread to several leaves on the same tree quickly. Within two or three weeks of initial infection, the fungus can be found on as many as 30 leaves in 100.

Germination

Once it settles on the underside of a coffee leaf, the fungus will produce 4 germ tubes over a period of 6 to 12 hours. These tubes grow until they reach the leaf stomata. From there, the fungus will require water, low light and temperatures below 82.4 degrees Fahrenheit.

Colonization

Once the fungus has penetrated the leaf, it begins to extract nutrients. Plant cells that have been parasitized lose their green coloration and begin to look yellowish. This stage can last from 21 to 24 days in the sun, or 18 to 22 days in the shade.

Reproduction

After 30 days of colonization, the fungus will be mature enough to start the cycle again. The fungus is polycyclic, meaning it can produce spores and reinfect plants on any given day throughout a growing season.

Symptom and Damage of Coffee Leaf Rust

The first symptoms of coffee leaf rust disease is small discolored spots which develop on the underside of the leaves. These small spots increase in size and are powdered with spores of the pathogen ranging in color from yellowish orange to bright orange (Muller *etal.*, 2004). On the upper surface of the leaves, the lesions are less conspicuous but on lower side of the leaves the lesions increase in size depending on the growth of the fungus inside the leaf (Kushalappa, 1989).

Coffee leaf rust affect plant growth by reducing the amount of leaf area available for photosynthesis, either by occupying leaf area or by inducing defoliation principally of the attacked leaves (Kushalappa, 1989). It brings loss of physiological activities in the affected part of the leaves and cause leaves to fall (Muller *et al.*, 2004). Potent attack of the disease can cause branches to wither completely and this hinders the plant or even stops its development. If the leaves are unable to supply the needs of the developing coffee berries, which act as powerful sinks, then they draw on the carbohydrate reserves of the roots and stems (Wrigley, 1988). Subsequently, badly diseased and weakened coffee plants do not survive (Muller *et al*., 2004). Depending on the severity of the coffee leaf rust, not only fewer flowers are formed but also the flowers and fruits formed fall prematurely and the remaining fruits often do not reach the maximum size; hence, causing reduction in both weight and volume of yield. The lower bean yield and poor bean quality in turn result from sever leaf fall and the general debilitating effect of coffee leaf rust on the tree (Bock, 1962b; Mayne, 1971).

Gene –for-Gene Resistance

An important class of genes for resistance to obligate bio-trophs has specific "gene-for-gene" interactions with pathogen genotypes. In a typical gene-for-gene relationship, the host is able to mount a successful defense against the pathogen if it has a resistance gene that corresponds to a specific pathogen avirulence gene. Phenotypic data indicate that gene-for-gene relationships operate in diseases caused by bacteria, viruses, insects, and nematodes, as well as fungi, but the number of cases in which this has been proven by genetic analysis is relatively small.), such that just one mutation may cause a pathogen to become virulent.("Virulence" is used here, as usual in plant pathology, to mean the qualitative ability of a pathogen to cause disease on a specific plant genotype.) on a host with the matching resistance gene.

At the moment, resistance genes could be divided in nine classes, denominated as SH1 to SH9, that isolated or in combination, result in resistance to different leaf coffee rust disease. Pathogen virulence is codified by the genes *v1* to *v9* (Bettencourt & Rodrigues 1988). Between resistance genes, *SH1*, *SH2*, *SH4* e *SH5* could be found in *C. Arabica* genotype. The genes *SH6*, *SH7*, *SH8*, *SH9* and others unknown, were introduced from *C. canephora*, and *SH3* from *C.*

liberica (Bettencourt e Rodrigues 1988; Prakash et al. 2004). At the present, are known 45 physiological races of *H. vastratrix* that are able to infect different genotypes (Várzea e Marques, 2005; Mahé et al., 2007). Nowadays, new molecular techniques allow us to quickly identify and characterize plant resistance genes, making more feasible the pyramiding of several resistance genes. Actually, is still very limited the number of molecular markers linked to coffee resistance. The locus *SH3* originated from introgression of *C. liberica* into *C. arabica*. Was fined mapped by Prakash et al. (2004), and characterized by Mahé et al. (2008). However, the physiological races of *H. vastratix* in Brazil have already overcome the *SH3* resistance gene, making the available markers not usefull to breeding purposes in Brazil. Previous work of your group (Brito ,2007), identified three markers linked to the single resistance gene.

Breeding Approach for Coffee leaf rust Resistance

Breeding for resistance to CLR took into consideration the worldwide distribution of the disease and the multiple races of the pathogen. Resistance to CLR is inferred from Flor's Gene-for-Gene concept, which states that for every major gene-conditioning resistance in the plant, there is a corresponding gene-conditioning virulence in the pathogen (Flor H. 1971). The most notable variety that was introduced in most countries was the Colombian Catimor, combining coffee leaf rust and coffee berry disease resistance and compact growth.

In Arabica coffee, vertical (complete), horizontal (race non-specific) and incomplete (partial) resistance to the leaf rust disease was reported (Rodrigues *et al*., 1975; Eskes, 1989a; Vàrzea *et al*., 2005). Complete resistance inhibits the infection process and prevent production of inoculum while the partial resistance which may also called incomplete resistance does not inhibit the infection process completely but allow the production of certain inoculum (Frantzen, 2000) through increased latency period and reduced lesion density. Horizontal resistance to coffee leaf rust aim at reducing the intensity of the attack and lengthening of the latency period, thus reducing the sporulation of the pathogen(Muller *et al.*, 2004). Consequently, it delays the epidemic and reduces the disease level in a population.

The rapid plant cell death at the infection site (hypersensitive reaction) is the most common interaction of incompatibility of gene for gene interactions. Resistance mechanism with hypersensitive response appeared to be efficient particularly against biotrophic pathogens, such as rust fungi, which depend on living host cells for their reproduction (Heath, 1981). Cytological and biochemical studies have shown that coffee cultivars display a hypersensitive response to the leaf rust associated with callose deposition, haustoria incasement, deposition of phenolic like compounds and host cell wall lignifications (Martins and Moraes, 1996; Silva *et al*., 2002).

Since devastation of the coffee industry in Sir Lanka and eventual introduction of rust to Asian and Latin American continents, great effort made to develop resistant varieties and the first Arabica coffee selection showing resistance was discovered in India. Sadly, when introduced into other areas its resistance failed due to existence of new physiological race with differing pathogenecity (Wrigley, 1988). With continued breeding efforts, the tetraploid genotypes known as Hibrido de Timor (HTD), derived from a spontaneous interspecific cross between *C. arabica* and *C. canephora (*Lashermes *et al.*, 2000*)* has been discovered and found resistant to CLR (Wrigley, 1988; Muller *et al*., 2004). These materials showed high level of resistance to all races of rust existed in Kenya (Waller, 1982) and Brazil (Carvalho *et al.*, 1989). Some of these lines were also introduced to Ethiopia from Portugal in 1979 and tested across locations *viz.* Tepi, Bebeka and Metu and the best two lines (Catimor J19 and Catimor J21) were released for production in low land areas. At that time, these lines conferred complete resistance to rust at all locations (Bayetta *et al.*, 1999) although they were not stringently tested.

In Ethiopia, the existence of six physiologic races in different coffee growing regions of the country was reported (Meseret *et al*., 1987). According to this report, race III was the most dominant (52.7%) and mostly prevalent in southwest forest coffee regions followed by race II which is distributed in all the areas where rust existed and in garden and plantation areas. Recent reports also confirmed the existence of races III and X in the forest coffee at Bonga and race II, at Berhane-Kontir in Ethiopia (Hindorf and Arega, 2006). Due to such proliferation of races able to overcome major gene resistance breeding strategies has to look for alternative durable resistance.

In Ethiopia, large genetic diversity of *C. arabica*, high level of horizontal (non-specific) resistance to CLR (Van der Graaff, 1981) and availability

of at least some incomplete resistance might likely protects coffee against rust under prevailing conditions (Meseret, 1983; Eskes, 1989a). Meseret (1996) identified coffee plants with partially (incomplete) resistance to CLR from lowland forest coffee of southwestern Ethiopia. Muller *et al.(*2004) also indicated an evidence for the existence of general resistance type (nonspecific) in wild genotypes *of C. arabica*. The presences of such wide range of resistance to CLR in wild forest coffee population provide an opportunity to develop and use resistant materials for CLR management, but yet unexploited so far.

Histological observations have shown that C. arabica resistance is expressed by a hypersensitive response (HR) with cell death of stomatal and mesophyll cells (Martins and Moraes, 1996; Silva et al., 2002, 2008). Molecular analyses have indicated that perception of the fungus, whether virulent or avirulent, occurs when the pathogen enters the stomata (Ganesh et al., 2006; Ramiro et al., 2009). However, specific host resistance responses, including hypersensitive cell death, H2O2 production and defence-related gene expression patterns, are associated with the production of haustoria in mesophyll cells, and not with the production of haustoria in stomatal cells, suggesting that specific recognition of H. vastatrix occurs at a later stage (Ramiro et al., 2009).

Recently, major insights have emerged from studies on biotrophic fungi, indicating that they secrete effector proteins, including virulence and avirulence proteins, that alter host physiology and defence responses (for review, see Stergiopoulos and de Wit, 2009). Effector proteins may be subdivided into two broad categories depending on whether they are secreted in the apoplasm or delivered into the cytoplasm of the host cell.

Until recently, the identification of in planta-expressed transcripts was a major limitation to studies of biotrophic pathogens. Sequencing through the Sanger approach has led to the identification of several hundreds of genes and a few secreted proteins (Joly et al., 2010; Puthoff et al., 2008; Yin et al., 2009). The fundamental knowledge of the functional genome is now being enhanced by the ability to deeply probe an organism's transcriptome using high-throughput sequencing data production.

The coffee genotypes are classified in physiological groups which are distinguished from each other essentially by responses involving either complete resistance or susceptibility (low and high infection type) to several rust races. Group A, characterized by resistance to all the known rust races, has been found in hybrids between *C. arabica* x *C. canephora*, either spontaneously as in the HDT or man-made as in Icatú (D'Oliveira and Rodrigues Jr. 1961; Marques and Bettencourt 1979).

Since 1927 the Central Coffee Research Institute (CCRI) in Balehonnur, in India, has carried out an important national breeding program. However, it was after the creation of CIFC that coffee breeding for rust resistance received a decisive impulse.

A major breakthrough in the CIFC's plant breeding program for obtaining resistant varieties was the discovery in the late 1950s of the Hibrído de Timor (HDT), a single plant found in the ex-Portuguese colony of Timor (now Timor Lorosae). Remarkably, most of the HDT offspring offered resistance to all or some of the known rust races. It has been demonstrated that HDT is supposed to be a natural hybrid between *C. arabica* and *C. canephora* and to have received from the latter the genes responsible for rust resistance. This interspecific hybrid has the same number of chromosomes as *C. arabica* and the crosses are fertile. The breeding programme of CIFC was essentially based on the utilization of HDT as a resistant parent. The main hybrids produced at CIFC with HDT were: HW26 = Caturra Vermelho x HDT 832/1; H 46 = Caturra Vermelho x HDT 832/2; H361 = Villa Sarchi x HDT 832/2; H528 = Catuaí Amarelo x HW26/13; H529 = Caturra Amarelo x H361/3.

Durability of Resistance

The fundamental and applied research developed at CIFC on leaf rust, as well as the research it originated in several coffee-growing countries, led to substantial advances towards obtaining durable resistance to this disease in Arabica. This progress has been possible as a result of a joint co-operation with several coffee Experimental Centers in different countries, namely Kenya (Coffee Research Station), Brazil (Instituto Agronômico de Campinas, Instituto Brasileiro do Café, Sistema Estadual de Pesquisa Agropecuária de Minas Gerais, Instituto Agronômico do Paraná, Universidade Federal de Viçosa and Universidade Federal de Lavras); Colombia (Centro Nacional de Investigaciones de Café); Central America (Mexico, Panama and the Dominican Republic) under the project Promecafe (PROMECAFE: Programa Cooperativo para la Protección, Modernización de la Caficultura en Centroamerica, México, Panama Y República Dominicana)

(Bettencourt, 1981; Bettencourt, 1983; Rodrigues Jr. et al., 2000).

In the last few years, some improved commercial varieties from HDT and other interspecific tetraploid hybrids, like Icatú are gradually losing their resistance to leaf rust in some countries, due to the appearance of new virulent races (Rodrigues et al., 2000; Várzea et al., 2004; Várzea and Marques, 2005). Some genotypes of the referred coffee varieties, however, maintain their resistance and others, although infected in the field, present an incomplete type of resistance with others heavily infected, suggesting that they probably possess a polygenic type of resistance, like the variety Colombia (Alvarado, 2005). On the other hand, some Arabica varieties like Rume Sudan and Tafarikella with low yields and classified at CIFC as belonging to the susceptible group E, showed a very high partial resistance in the field for many years (Várzea et al., 2000; Várzea et al., 2002a). At CIFC the level of resistance of HDT derivatives and some lines of Rume Sudan are now being re-evaluated. However, it is interesting to note that when the yield is totally suppressed, the susceptible cultivar Caturra appears partially resistant under conditions of strong infection (Bertrand et al., unpublished data). Moreover, in a F2 population resulting from a cross between 'Resistant x Susceptible', the same authors observed that plants with low productivity appear with very high frequency, partially or totally resistant and plants with high productivity appeared resistant or susceptible and rarely partially resistant. Consequently, some partial resistance might be explained by the physiological status of the plant.

Cytological and Biochemical Resistance Mechanisms

There is no evidence for the existence of preformed defences in coffee, which could limit the growth of *H. vastatrix* although several resistance mechanisms are induced after infection (Rodrigues et al., 1975; Kushalappa and Eskes, 1989; Rodrigues et al., 2000; Várzea et al., 2002a, Várzea et al., 2004). *H. vastatrix* urediospores usually germinate and differentiate the appressoria over stomata equally well on susceptible and resistant coffee plants (Silva 1996; Silva, et al., 2002). For a number of coffee (*Coffea* spp.) genotypes, the resistance is post-haustorial (in that the fungus ceases its growth at different stages of the infection, but more frequently after the formation of the first haustorium) and is expressed by the rapid hypersensitive cell death (HR)recognized by the presence of autofluorescent and/or browning cells or by deep blue staining with Evans blue. Cell death began to be observed around 2 days post-inoculation, in the guard cells only, or in both the guard and subsidiary cells at the infection sites in which the fungus reached the stage of appressorium or penetration hypha (Silva 1996; Silva et al., 2000, 2002). Death of subsidiary and mesophyll cells invaded by a haustorium was observed from the 3rd day after inoculation. During the time course of infection, cell death spread to adjacent epidermal and mesophyll non-invaded cells, as has been generally described for other coffee resistant genotypes (Martins et al., 1985; Rijo et al., 1991) and in plants resistant to other rust fungi and to other obligate biotrophs (Heath, 2000a, Koga et al., 1990; Huang et al., 1998). In susceptible coffee plants, death of guard and subsidiary cells was observed from the 3rd day after inoculation, but only in a small percentage of infection sites (generally less than 20 %), in which the fungus had stopped its growth at early stages (Silva, 1996; Silva et al., 2002). Transmission electron microscope observation of host cells undergoing HR revealed membrane breakdown at the level of the plasma membrane and in different organelles, namely chloroplasts, nucleus and mitochondria, with a change in the chloroplast and nucleus appearance and coagulation of cytoplasm (Silva et al., unpublished data).

Another signal of incompatibility detected early at the cytological level was the haustoria encasement. This host response was also observed in compatible interactions, but latter in the infection process (from the 7th day post-inoculation) and only in a small number of haustoria (Silva, 1996; Silva et al., 1999a, 2002). The haustoria encased material in resistant or susceptible leaves reacted positively for callose and □-1,4-glucans, as indicated by the use of polyclonal antibodies raised against □-1,3-glucans and an exoglucanase-gold complex, respectively. The use of anti-galacturonic acid monoclonal antibodies (JIM7) allowed the localization of pectins in the encasing material around the penetration pegs, but not around haustorial bodies (Silva et al., 1999a, 2002). In several plants resistant to rust and other obligate biotrophs, haustorium encasement has been regarded as one expression of incompatibility (Littlefield and Heath 1979; Skalamera et al., 1997). Callose, the major compound of haustorial encasements, has been reported to be less permeable to small molecules

than other cell wall components (Heslop-Harrison, 1966) and may therefore restrict the passage of nutrients to the fungus and consequently to slow the fungal growth (Rijo and Vasconcelos, 1984).

In resistant coffee leaves, the early detected epifluorescence and/or browning of cells was followed by the lignification of their walls, which occurred from the 7th day post-inoculation, as indicated by the phloroglucinol-HCl test (Rijo and Vasconcelos, 1984; Martins et al., 1985; Silva et al., 2002). Biochemical studies, with coffee resistant genotypes, revealed an early increase of phenylalanine ammonia-lyase (PAL) and peroxidase activity just before or at the same time as the beginning of the observation of cell death, which may indicate the involvement of these enzymes in HR. By 4-5 days post-inoculation a second increase of PAL and peroxidase activity was observed which can be related with the later accumulation of phenolic compounds and lignification of the host cell walls detected cytologically (Silva et al., 2002, 2003a, b). The isoenzyme pattern for peroxidases obtained by IEF gels showed an increase in activity of anionic isoenzymes and a new cationic isoform at the same time as the first peak of peroxidase activity detected in the incompatible interactions (Silva et al., 2003a, b). At that time, peroxidase activity, cytochemically localized using DAB (Diaminobenzidine), was detected at the interface host cuticle-fungal pre-penetration structures, as well as in the walls, middle lamella, cytoplasmic contents, chloroplasts and endoplasmic reticulum of stomatal and spongy cells, at infection and penetration sites. The treatment of resistant coffee leaves with 2,4-dichlorophenol, an activator of peroxidases and other oxidases, significantly increased cell death. On the contrary, salicyl hydroxamic acid, an inhibitor of the same enzymes and diphenyleneiodonium chloride, an inhibitor of NADPH oxidases decreased cell death. These results suggested that the peroxidases, NADPH oxidases and eventually other oxidases are involved in the HR of the coffee-rust interaction. The same kind of treatments using scavengers of active oxygen species (catalase, superoxide dismutase and manitol) showed that only the superoxide dismutase significantly inhibited the cell death, also suggesting the involvement of the superoxide anion radical O_2^- in the HR (Silva et al., 2001, 2003b). On the other hand, studies made by Rojas et al. (1993) revealed a rise in lipoxygenase activity during an incompatible coffee-rust interaction, whereas the activity of the enzyme remained fairly constant in the compatible interaction.

An early increase of chitinase and glucanase activity in coffee-leaf rust incompatible interactions, but not in the compatible ones was observed by Maxemiuc-Naccache et al. *(*1992) using crude extracts of coffee leaves. Similar results were obtained when studying chitinase activity in intercellular fluids (IF) of incompatible coffee-rust interactions. Although basic isoforms of chitinases, from IF of coffee leaves, were present in both compatible and incompatible interactions, they were detected earlier in the incompatible ones. Immunodetection analyses performed with antibodies specific to class I chitinases revealed the importance of these isoforms in the incompatible interactions (Guerra-Guimarães et al., 2001, 2003).

Ultrastructural observations of different coffee resistant genotypes revealed the accumulation of a material partially crystallised in the intercellular spaces around the senescent hyphae, next to dead host cells and in close association with the middle lamella, around 5-7 days post-inoculation. However, such material was never detected in healthy or susceptible tissue. Cyto- and immunocytochemical tests showed that at the beginning of accumulation the material contained weakly esterified pectins. It also contained polysaccharides and phenolic-like compounds. Cellulose, hemicellulose, extensins, hydroxyproline -rich glycoproteins and proteins were not detected. Although the role of this material is unknown it might be the result of plant cell death associated with the slowdown of tissue invasion by the pathogen (Silva et al., 2002, 2005). Another response observed, around 12 days post-inoculation in different coffee resistant genotypes was the hypertrophy of the host mesophyll cells in the infection area (Rijo et al., 1990; Silva et al., 2002) suggesting the possible involvement of growth regulators. These larger cells surrounding the intercellular hyphae gave rise to a localized tumefaction and corresponded macroscopically to the reaction type *flt*, the most common reaction type of incompatible coffee-rust interactions.

Molecular Approaches to Coffee Breeding

To gain insights into defence and resistance gene activation in coffee, a catalogue of genes expressed early in coffee leaves when challenged by the rust pathogen was established (Fernandez et al., 2004; Santos et al., 2004). Two subtractive cDNA libraries were constructed and Expressed Sequence Tags (ESTs) were generated by

random sequencing of cDNA clones. Library 1 contained subtracted cDNAs obtained from coffee leaves inoculated with the rust fungus for 12 h. Library 2 contained subtracted cDNAs derived from a pool of mRNAs obtained from coffee leaves collected 24 and 48 h post-inoculation (Fernandez et al., 2004). Genes associated with expression of early resistance mechanisms of coffee plants to **parasites were** isolated from the two cDNA libraries (Fernandez et al., 2004). At least 13 % of the ESTs may represent genes involved in plant defence reactions (disease resistance proteins, stress- and defence-proteins, components of resistance signal pathways), 13 % in cell signalling processes (ionic channels, MAP kinases, receptor kinases), and 13 % in gene regulation (transcription factors, proteasome machinery). The highest proportion of cDNAs (34 %) were homologous to plant genes of unknown function and might be an additional source of genes participating in the expression of coffee defence responses to parasites. Although far from being exhaustive, the ESTs isolated may provide a significant set of data for improving our knowledge of coffee resistance to pathogens. A lot of the genes isolated showed homology to known plant genes suggesting conservation of signalling pathways and resistance mechanisms against pathogens in *C. arabica* and other plants.

Finally, 4 other HR-upregulated cDNA clones were of potential interest regarding defence mechanisms. One EST putatively encoded a receptor-like kinase. This class of signal proteins is involved in a diverse array of developmental and defence functions (Du and Chen, 2000; Morris and Walker, 2003). Another gene best matched an UDP-glucose: salicylic acid glucosyltransferase. Glucosyltransferases catalyze the transfer of glucose residues to numerous substrates and regulate the activity of compounds that play important roles in plant defence against pathogens, such as salicylic acid (Chong et al., 2002). Two ESTs putatively encoded an AP2-type transcription factor and a WRKY transcription factor. A number of gain-of-function studies have shown the direct implication of several transcription factors in potentiating the plant responses to pathogen infection. Particularly involved are several WRKY proteins which are implicated in the regulation of several biological processes, including pathogen defence (Dong et al., 2003; Ülker and Somssich, 2004).

Coffee gene induction that we observed around 12-18 hpi shows that recognition of the pathogen may occur soon after penetration of the fungus into the substomatal chamber. In several plant-rust interactions, host specific resistance responses are typically expressed concurrently with the formation of the first haustorium (Heath, 1997b; Mould et al., 2003).

In the coffee–*H. vastatrix* interaction, the haustorium stage may be reached by *H. vastatrix* between 24-48 hpi (Martins and Moraes 1996; Silva et al., 1999a, 2002). Cytological observations of resistant coffee leaves revealed that in many infection sites (stomata) the fungus had stopped its growth at a pre-haustorium stage (HMC) (Silva *et al*., 2002), suggesting that early host resistance responses may be expressed. The gene activation observed around 12-18 hpi may be part of the coffee resistance responses and may determine the outcome of the coffee-rust interaction.

Efficient and reliable disease screening methods are required for a successful variety development programme. Molecular markers linked to resistance provide the potential to screen for resistance in a large population of plants at any stage of plant development. Where several genes confer resistance, markers have the advantage over morphological assessments, because plants carrying multiple resistance (broad-based resistance) can easily be differentiated from those carrying a single gene (narrow-based resistance)(Gichuru *et al*, 2008; Omondi *et al* ,2009).

SUMMARY AND CONCLUSION

Coffee Leaf Rust (CLR) is one of the most important diseases of C. arabica in the world, yield reductions of 20% or more in various countries are still caused by the pathogen. Coffee leaf rust was first reported in 1934 in Ethiopia but it has never reached to epidemic level to cause eradication of Arabica coffee. This may be as a consequence of long-term coexistence of rust and coffee which created a balanced pathosystem and high level of horizontal (race nonspecific) resistance. Currently, coffee leaf rust is widely distributed all over coffee growing regions of the country with varying intensities. Highest diseased trees with mean percent of 42.5 in Kaffa, 41.9 in Illuababor, and 39.6 in Hararghe. The average national infected trees were estimated to 12.9% in 1980 and increased by threefold (36.3%) after ten years in 1990.The first symptoms of coffee leaf rust disease is small discolored spots which develop on the underside of the leaves. These small spots increase in size and are powdered with spores of the pathogen ranging in color from yellowish orange to bright orange. Coffee leaf rust affect

plant growth by reducing the amount of leaf area available for photosynthesis, either by occupying leaf area or by inducing defoliation principally of the attacked leaves. Between resistance genes, *SH1*, *SH2*, *SH4* e *SH5* could be found in *C. Arabica* genotype. The genes *SH6*, *SH7*, *SH8*, *SH9* and others unknown, were introduced from *C. canephora*, and *SH3* from *C. liberica.* Race III was the most dominant (52.7%) and mostly prevalent in southwest forest coffee regions followed by race II which is distributed in all the areas where rust existed and in garden and plantation areas. Recent reports also confirmed the existence of races III and X in the forest coffee at Bonga and race II, at Berhane-Kontir in Ethiopia. There are different resistance breeding approaches; durable resistant methods, Cytological and biochemical resistance mechanisms, Molecular approaches to coffee breeding and other hybridization methods and pure line methods were used today resistant breeding program.In Ethiopia, large genetic diversity of *C. arabica*, high level of horizontal (non-specific) resistance to coffee leaf rust and availability of at least some incomplete resistance might likely protects coffee against rust under prevailing conditions identified coffee plants with partially (incomplete) resistance to coffee leaf rust from lowland forest coffee of southwestern Ethiopia. The presences of such wide range of resistance to coffee leaf rust in wild forest coffee population provide an opportunity to develop and use resistant materials for coffee leaf rust management, but yet unexploited so far.

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