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Diversity in Pathogenicity and Genetics of Gibberella xylarioides (Fusarium xylarioides) Populations and Resistance of Coffea spp. in Ethiopia

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Diversity in Pathogenicity and Genetics of *Gibberella xylarioides* (*Fusarium xylarioides*) Populations and Resistance of *Coffea* spp. in Ethiopia

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1 INTRODUCTION

Ethiopia is the country with the longest tradition of coffee production and consumption in the world. Coffee consuming is well embedded in the daily life of almost all Ethiopians, and thus the crop is socially as well as economically important to the country. In general, almost half of the coffee produced in the country is consumed locally. The domestic consumption level was 48.9% of the total production in 2003, the largest in the continent and 8th in the world (ICO 2003). This cultural heritage, nevertheless, significantly contributed to a sustainable production of the crop for centuries in Ethiopia. Ethiopian coffee is empirically organic by world standard owing to its ecological and traditional way of exploitation although not yet fully certified (Taye and Tesfaye 2001). Besides there are famous localities such as Harar, Jimma, Limu, Nekemet, Sidamo and Yirgacheffe, that are specifically known for premium coffee quality in the world market. Coffee is still the number one cash crop for most subsistence farmers, and yet with the current price slump in the global market it accounts for over half of all earnings from merchandise exports of the country.

Arabica coffee (*Coffea arabica* L.) is the only species produced in Ethiopia although the introduced Robusta coffee (*Coffea canephora* Pierre) is grown in some research plots and in a few hectares at Tepi and Bebeka plantations. Coffee production in this country is broadly grouped on the basis of biological diversity of the species and level of management into four systems, namely; forest, semiforest or semidomesticated, garden and plantation coffee (Meyer 1965, IAR 1971, Paulos and Demel 2000).

Forest coffee, which sometimes referred to as ‘wild coffee’, regenerates spontaneously from self-sown seedlings as understory of intact multilayered tropical rainforests. This system is characterized by multifarious Arabica coffee gene pools accompanied by enormous biological diversity of the flora and fauna situated in the west and the southwest of Ethiopia. Semiforest or semidomesticated coffee is simply derived from the wild forest coffee by human intervention and domestication, through thinning the dense overstory of the forest trees and slashing the understory bushes and shrubs. The open areas remained after the removal of the lower strata are filled mostly by transplanting naturally perpetuating coffee seedlings under the mother trees

resulting in irregularly spaced high population density. Weeding by slashing is the only management practice applied once a year just before or during harvesting season to facilitate picking the crop. This system is also believed to include many landraces and contributes to a greater proportion of 35 – 40% to the total production. Although this system is largely confined to the west and southwest of the country, semidomesticated coffee occurs also in the south but with more management, low shade intensity and comparatively less heterogeneity in the host composition.

The garden coffee production system is predominant in the southern region of Sidamo areas, in the east of the Hararge province and in some minor coffee growing regions. The system has a varying size of less than 0.5 ha of coffee plots around the farmer's dwellings usually intercropped with different food crops. In Hararge, sorghum (*Sorghum* spp.), maize (*Zea mays*), sweet potato (*Ipomoea batatas*) and chat (*Catha edulis*) are used, while in the south enset (*Ensete ventricosum*) and some fruit trees like banana (*Musa* spp.), papaya (*Carica papaya*), mango (*Mangifera indica*) and avocado (*Persea americana*) are dominantly intercropped with coffee. The coffee population is less diverse because the crop could be selectively developed by farmers, and more intensive agronomic practices such as slashing and hoeing (3 – 5 times per year) are employed, and to some extent trees are pruned, mulched and manured (Workafes and Kassu 2000).

Plantation coffee is of young history in the aged tradition of coffee cultivation in Ethiopia. This system is established by transplanting seedlings, raised in nurseries from desirable cultivars with high yield and disease resistance, into well prepared land of cleared forest trees and shrubs including the local coffee bushes. Owners of these estates are commercial enterprises such as Bebeka, Tepi and Limu Coffee Plantation Development Enterprises and sometimes some small-scale farmers. Currently, there are private companies or investors establishing large commercialized coffee farms. The system involves monoculture-based high input/output and market-oriented approaches with year round intensive management adopting recommended technologies with limited cultivars, row planting, proper spacing, mulching, pruning, use of herbicides and fertilizers. Crop yields are at least two-fold higher than the traditional ones, howsoever, the area accounts about 5% of the overall production.

About 80% of the coffee traded in the world is Arabica coffee and the remaining 20% is Robusta. Arabica coffee, which is the only self-fertile tetraploid species of the genus *Coffea*, produces superior quality coffee. However, this species is also susceptible to most diseases, insects as well as root-knot nematodes as compared to all other *Coffea* spp. (van der Graaff 1983, Bertrand et al. 2001). The crop is seriously damaged by coffee berry disease, *Colletotrichum kahawae*; leaf rust, *Hemileia vastatrix* and coffee wilt, *Gibberella xylarioides* (Walyaro 1997, Hindorf 1998). Coffee berry disease (CBD), the most serious disease of Arabica coffee, causes on average about 30% national yield losses to Ethiopia (Tefesetewold 1995, Eshetu et al. 2000). Coffee leaf rust (CLR) occurs in Ethiopia at tolerable levels unlike other countries, as the pathogen exists under a balanced pathosystem, and it inflicts minor attack to the crop except in certain areas in Hararge and some pocket fields planted with homogeneously susceptible cultivars at lower elevations (Meseret et al. 1987, Eshetu et al. 2000).

Coffee wilt disease (CWD), also known as tracheomycosis, is perhaps historically the first renowned coffee disease in causing large-scale damage to various coffee plantation in Africa in the early 1950s (Wellman 1961, Muller 1997). The disease was first observed on Excelsa coffee (*Coffea excelsa*) in 1927 and reported to decimate over 280 ha of Excelsa plantation in the Central African Republic alone. During the same period the disease destroyed thousands of hectares of several varieties of Robusta coffee in Ivory Coast, Cameroon and Zaire (Congo) (Wellman 1961, Booth 1971, Muller 1997). The survey report of Kranz (1962) indicated that Kouillu and Game varieties of Robusta coffee were wiped out in Guinea.

The disease represents a typical vascular wilt specific to coffee, incited by a fungal pathogen *Gibberella xylarioides* Heim & Saccas (anamorph: *Fusarium xylarioides* Steyaert). The biology and epidemiology of *Gibberella xylarioides* has been little understood. It was considered to be a soil inhabiting fungus which penetrates coffee tree through wounds in the aerial parts and the roots. After slow incubation period, the pathogen finally invades the vascular tissues resulting in rapid wilt and death of the tree (Flood and Brayford 1997, quoting Jacques-Felix 1954). The symptoms of infection are epinasty of leaves followed by drying and defoliation, with certain discolouration under the bark (van der Graaff and Pieters 1978, Flood 1997, Girma et al. 2001).

After remaining an economically less important disease for three decades, tracheomycosis re-emerged as a serious disease of coffee in Africa (Flood and Brayford 1997). The first new outbreak was noted on Robusta coffee in Zaire (Congo) in the mid of 1980s (Mfwidi-Nitu 1994) and further surveys carried out by Flood (1996) ascertained that the disease claimed considerable losses estimated up to 90% in the north-east of that country. In Uganda, where it was unknown hitherto, the disease is noticed in 1993 in two Robusta growing districts that border Zaire and rapidly spread to 12 districts within the next 3 years (Flood 1997, Lukwago and Birikunzira 1997). The prevalence of CWD in 22 of the 30 coffee growing districts was lately confirmed with incidence ranging up to 40%. The worst attack occurred in 4 districts, where on average 40 to 50% of the coffee fields are affected. Since its first outbreak in 1993, the disease has destroyed about 9 million coffee trees throughout Uganda (CORI 2001). Coffee wilt is reported to spread to Tanzania most probably from Uganda, but not yet discovered in Rwanda, Malawi and Kenya (Kilambo et al. 1997, Hillocks et al. 1999).

In Ethiopia, the earliest documented account of the disease was by Stewart (1957), who first described the wilting syndrome and attributed the cause to *F. oxysporum* f. sp. *coffea*. Some 15 years later Kranz and Mogk (1973) authenticated the first record of tracheomycosis and its causal pathogen on Arabica coffee as a new host in Ethiopia. Initial as well as subsequent surveys accompanied by isolation works verified the occurrence of the disease in most coffee growing areas of the country (van der Graaff and Pieters 1978, Merdassa 1986, Girma 1997). During the latest time, CWD has been remarkably increasing outbreaks throughout the major coffee producing districts in the south and south-west of Ethiopia (Girma et al. 2001). Two main factors, acting either independently or in concert, have been postulated about the re-emergence of CWD in Africa and becoming a major constraint to coffee production in the continent. The former effective host resistance to control the disease for some three decades might have been broken due to resurgence of new aggressive and virulent pathogen strains and/or the recent prevailing environmental conditions and production systems might have favoured the multiplication and dissemination of the fungus (Flood 1996, Flood and Brayford 1997, Girma and Hindorf 2001).

By analyzing the structure of the pathogen populations it is possible to understand the mechanisms of a change in populations, and this understanding provides the basis for disease management

strategies including development and use of resistant hosts (Leung et al. 1993). Population structure, as well defined by Leung et al. (1993), McDonald (1997) and McDonald and Linde (2002), refers to the amount of genetic variation among individuals in a population, the ways in which this variation is partitioned in time and space, and the phylogenetic relationships among individuals within and between sub-populations. The most widely used method of characterizing pathogen populations is the determination of the virulence spectrum on a set of differential varieties carrying different resistance factors. However, for certain reasons a population structure inferred from virulence data may not reflect the true genetic diversity and evolutionary history of the isolates examined. Thus, population structure should be inferred from a variety of neutral markers distributed randomly in the genome (Leung et al. 1993, Kistler 1997, McDonald 1997).

Random amplified polymorphic DNA (RAPD)-PCR analysis has been used extensively to define fungal populations at species, infraspecific, race and strain levels including *Fusarium* spp. (Assigbetse et al. 1994, Bentley et al. 1995, Bentley and Bassam 1996, Bentley et al. 1998, Viljoen et al. 1997, Migheli et al. 1998), *Phythophthora* (Hantula et al. 2000) and *Rhizoctonia* (Banniza et al. 1997). The RAPD fingerprinting assay detects small inverted nucleotide sequence repeats throughout the genomic DNA. It involves only a single primer of arbitrary nucleotide sequence that binds to the genomic DNA on two different priming sites in an inverted orientation, and amplification between these points results in a discrete products. As each primer can be expected to amplify several discrete loci in the genome, the final result is generally a profile of amplification products of varying sizes (Edel 1998, Bridge and Arora 1998).

G. xylarioides was presumed to be a heterothallic ascomycete (Booth 1971), which is known to have a sexual or teleomorphic state in nature producing fertile perithecia in dead coffee plants (van der Graaff and Pieters 1978, Flood and Brayford 1997, Girma et al. 2001). Genetic diversity is most likely expected in such heterothallic organism, which usually form its sexual stage in nature where recombination through meiosis would generate a large number of unique genotypes (Chen and McDonald 1996). Sexual reproduction also results in ascospores that can function as overwintering structures or infective propagules and can be important component of the disease cycle (Glass and Kuldau 1992, Duncan et al. 1998). Thus, the major objectives of this study were: