# Breeding Tools for Durable Resistance to Nematodes (*Meloidogyne* spp.) of Coffee Varieties

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**Abstract**

Coffee is one of the world's largest traded commodities, produced in over 60 countries. Root-knot nematodes (*Meloidogyne* spp.) have become a major threat in all major coffee-growing areas. So far, more than fifteen species of *Meloidogyne* have been reported as pathogens of coffee (*Coffea arabica* L.). Nematocide treatments are only effective in the short term, expensive and environmentally hazardous. Growing nematode-resistant coffee trees constitutes so far the most promising option to control the pest. During the last decade, resistance to root-knot nematode have been identified in spontaneous accessions and relative diploid species. With the aim of improving the resistance to root-knot nematodes of coffee varieties grown in Latin America, a project was initiated in February 2002 with the financial support of the European Community (INCO, Contract ICA4-CT-2001-10070). The selected strategy relies upon the combined development of (i) varieties (either cultivar or rootstock) associating complementary well-characterised resistance genes, and (ii) optimised variety-deployment strategies based on a careful characterisation (geographical distribution, virulence and pathogenicity) of root-knot nematodes populations damaging coffee trees.

**Keywords**: nematode; rootstock; genetic; resistance; *Coffea arabica* L.

## INTRODUCTION

Coffee is one of the world's largest traded commodities, contributing over 7–12 billion US dollars annually. In particular, coffee represents one the key export and cash crops in tropical and subtropical developing countries with generally a favourable impact on the social and physical environment. Although the genus *Coffea* is reported to comprise over 80 species, only two species *Coffea arabica* L., popularly called as arabica, and *C. canephora* Pierre, known as robusta are under commercial cultivation. Arabica, the high land coffee accounts for nearly 70% of global production while robusta coffee is more adaptable for low lands and contributes remaining 30 percent.

*Coffea arabica* L. ($2n = 4x = 44$) is an allotetraploid species native to Africa containing two diploid genomes that originated from two different diploid wild ancestors ($2n = 22$), *C. canephora* and *C. eugenioides* (LASHERMES *et al.* 1999). *C. arabica* is characterised by a very low genetic diversity which is attributable to its origin and domestication (ANTHONY *et al.* 2002). In contrast, a considerable variability was reported among diploid coffee species (BERTHAUD & CHARRIER 1988). However, conventional coffee breeding methodology faces considerable difficulties when transferring resistance traits into Arabica cultivars (LASHERMES *et al.* 2000; VAN DER VOSSEN 2001). Combining various genes of resistance without reducing coffee quality appears as a very difficult task in an acceptable time-frame (FERNANDEZ & LASHERMES 2002).

Root-knot nematodes (*Meloidogyne* spp.) have become a major threat in all major coffee-growing (*Coffea arabica* L.) areas throughout the world (CAMPOS *et al.* 1990). In most Latin American countries, root-knot nematodes constitute the major agronomic constraint. In this report, aspects regarding protection of coffee plantings against nematodes are pointed out. Finally, a project initiated in 2002 with the financial support of the European Community (INCO, Contract ICA4-CT-2001-10070) is presented.

### High diversity of nematode populations

In association with differences in the virulence characteristics, a large diversity in terms of nematode species (*Meloidogyne* spp.) has been observed in coffee fields. More than fifteen species of *Meloidogyne* have been so far reported as pathogens of coffee (CAMPOS *et al.* 1990; CARNEIRO *et al.* 1996a). Regarding Latin America, 3 dominant species *M. exigua, M. incognita* races 1, 2, 3 and 4, and *M. paranaensis* have been described in Brazil (CARNEIRO *et al.* 1996a,b, 2001) while a contrasted picture has been reported in Central America (HERNÁNDEZ 1997). *M. exigua* is also present in most countries of Central America (Costa Rica, Nicaragua, Honduras) but surprisingly has not been observed in Guatemala. In that country the dominant species is still unidentified but highly similar to *M. paranaensis*. In Costa Rica, *M. arabicida* is responsible for severe dieback (BERTRAND *et al.* 2000a). In Salvador, three different types have been observed: *M. arenaria* and two unidentified populations. Furthermore, recent studies of isoenzyme systems have revealed intrapecific diversity (CARNEIRO & ALMEIDA 2001).

### The limitations of chemical control

Facing several obstacles and much debated, the use of nematocide treatments is declining (VILLAIN *et al.* 2002). Nematodes may have an adverse effect on soil biological activity. In addition, the soil, topographic and climatic conditions that characterise mots coffee production zones increase the risks of pollution. Furthermore, the efficacy of nematocide treatments appears very depending on the application frequency and the doses applied per plant. The high cost of nematocides often makes them inaccessible to small-scale growers, while in large and medium-size plantations, the recent coffee market crises has forced producers to optimise the production costs. Actually, current applications techniques are largely incompatible with the economic and ecological constraints. They should therefore be seen as a protective tool for use in purifying nursery substrates, pending the development of other effective methods for preventing nursery contamination.

### Several resistance genes identified

Resistance's to root-knot nematodes prevailing in Latin America were identified either in spontaneous accessions of *C. arabica* or in a relative diploid species, *C. canephora*. Resistances to *M. arabicida* (BERTRAND *et al.* 2000a,b), *M. incognita* from Guatemala (ANZUETO *et al.* 2001) and Brazil (GONÇALVES *et al.* 1996), and *M. paranaensis* (GONÇALVES *et al.* 1998) have been identified in Arabica spontaneous accessions. Furthermore, resistance sources to *M. exigua* have been identified in progenies derived from interspecific hybrids between *C. arabica* and *C. canephora* (GONÇALVES *et al.* 1996; GONÇALVES & PEREIRA 1998; BERTRAND *et al.* 2001). When studied through conventional genetic segregation analyses, the resistance to *M. arabicida, M. exigua* and *M. paranaensis* appeared to be due to the presence of major genes. A resistance to *M. exigua* transferred into *C. arabica* from *C. canephora* has been studied more precisely (NOIR *et al.* 2003). Segregation data analysis of a $F_2$ progeny showed that the resistance is controlled by a simply inherited major gene (designated the *Mex*-1 locus). Associated molecular markers were identified and a localised genetic map of the chromosome segment carrying *Mex*-1 was constructed. These results represent an important starting point to enhance backcross-breeding programmes and to perform an early selection of resistant seedlings.

### Resistance mechanisms largely unknown

Plants exhibit a wide range of defence mechanisms leading to expression of resistance to pathogens, including a wide range of nematodes. Although crucial to understand and to control the disease, elucidation of physiological mechanisms underlying ability of coffee tree to resist infection by nematodes has retained little attention. Preliminary histological studies of roots showing resistance to *Meloidogyne* indicated an abnormal development of the nematode feeding structure (i.e. misshapen giant cells) associated with adjacent localised death of cells (RODRIGUES *et al.* 2000; ANZUETO *et al.* 2001).

### Development of resistant coffee trees

In response to the phytosanitary problem due to nematodes, two complementary strategies are being conducted by the different coffee breeding programmes in Latin America: the development of rootstock and the selection of resistant Arabica cultivars. Although technically difficult to implement, grafting on nematode-resistant rootstocks is considered as the best solution in the short term. In a medium or long-term perspective, the availability of cultivars combining good quality, high yielding with nematode resistance appears as a crucial priority.

Grafting Arabica cultivar on *C. canephora* rootstocks has been a traditional method to reduce production losses due to nematodes in several Central American countries and Brazil. The rootstock seeds come from non selected trees and a large number of plants are susceptible to aggressive nematodes (BERTRAND *et al.* 2000b). A selection for the resistance to nematodes appears therefore indispensable. In addition, *C. canephora* rootstocks are not adapted to high elevation area where a large proportion of coffee trees is cultivated. Regarding the development of nematode-resistant Arabica cultivars, resistance genes to *M. exigua* have been successfully introgressed from *C. canephora*. Next step would be to combine these genes with complementary resistant-genes in order to get an adequate resistance spectrum.

## Ongoing project

The project entitled "Breeding tools for durable resistance to root-knot nematodes (*Meloidogyne* spp.) of coffee varieties in Latin America" involves partners from Europe [Institut de Recherche pour le Développement (F); Centro de Investigação das Ferrungens do Cafeeiro (P); Università di Trieste, Dip. di Biologia (I) ; Centre de Coopération International en Recherche Agronomique pour le Développement (F)], Central America [Centro Agronómico Tropical de Investigación y Enseñanza (CR); PROMECAFE network] and Brazil [Recursos Genéticos e Biotecnologia/ EMBRAPA; Centro de Café e Plantas tropicais/IAC]. The selected strategy for developing genetic durable resistance relies upon the combined development of (i) varieties (either cultivar or rootstock) associating complementary well-characterised resistance genes, and (ii) optimised variety-deployment strategies based on a careful characterisation of root-knot nematodes populations damaging coffee trees. The primary objectives are to:

- Characterise the root-knot nematode populations damaging cultivated coffee-trees (*Coffea arabica* L.) in Brazil and Central America.
- Establish the spectrum of resistance conferred by the available root-knot nematode resistance-genes identified in the coffee genetic resources.
- Determine the resistance phenotypes and defence mechanisms associated with different nematode resistance-genes.
- Extend and implement molecular marker-facilitated selection tools in relation to coffee breeding.
- Evaluate different rootstock types for nematode resistance, adaptation to various agro-ecological conditions and influence on coffee quality.

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