

Individual partner final reports
ICIA/IRTA: participant N^o4

1. Objectives

The objectives of Participant 4 were:

- Establishment and maintenance of a representative collection of AM fungal species *in vivo* on trap plant cultures.
- Study of the effects of early mycorrhizal inoculation in the growth and health of *in vitro* plantlets and their subsequent behaviour in the nursery.
- Effect of the mycorrhization of *in vitro* produced bananas and plantains on plant growth and health, under biotic stress conditions (nematode and fungi)

2. Activities

To achieve the above mentioned objectives, Participant 4 was involved in four Work Packages involving various activities:

WP1: Establishment of a collection of Arbuscular Mycorrhizal Fungi (AMF)

Three activities were conducted within this WP:

- a. Isolation and classification of arbuscular mycorrhizal fungi from Canary Island
- b. Establishment and maintenance of an *in vivo* collection of the arbuscular mycorrhizal fungi
- c. Supplying inocula to other participants involved in the project

WP3: AMF formulation and inoculation procedures for micropropagated bananas and plantains

Five activities were conducted within this WP:

- a. Studies of the effect of mycorrhizae on growth of the micropropagated banana plantlets during the acclimatization phase in the nursery
- b. Determination the effect or two different AMF by studying the morphological and topological aspects of root system and changes in development on two commercial banana cultivars
- c. Determination and comparison the ability of different AMF isolates (maintained in collection) on micropropagated banana plantlets during the first stages of development in the nursery
- d. Study the interaction between two beneficial microorganisms (AMF and PGPRs), on different commercial clones of *Musa*
- e. Study the effect of alginate entrapped *in vitro* produced *Glomus* strains on the first stages development of micropropagated banana plantlets

WP5: Interaction studies between mycorrhizal banana and plantains and their main root pathogens

Five activities were conducted within this WP:

- a. Evaluation the host suitability of four cultivars of local bananas against five isolates of lesion nematode *Pratylenchus goodeyi*
- b. Study the evolution of the root-knot nematode population *Meloidogyne javanica* in the presence of *Glomus manihotis* in banana

- c. Study the effect of the AMF *Glomus mosseae* on micropropagated banana infected with a high dose of *Fusarium oxysporum* f.sp. *cubense* (FOC)
- d. Study the response of micropropagated banana cultured under microplots conditions in soil infected with lesion nematode *Pratylenchus goodeyi*
- e. Study the combines effect of two beneficial microorganims: AMF and PGPRs on micropropagated banana infected with the root-knot nematode *Meloidogyne javanica*

3. Results achieved

WPI: Establishment of a collection of Arbuscular Mycorrhizal Fungi (AMF)

- a. Isolation and classification of arbuscular mycorrhizal fungi from Canary Island

Seven fungi from Canary Island have been isolated and classified :

Glomus mosseae (Nicol.& Gerd.) Gerdemann & Trappe
Glomus aggregatum Schenck & Smith
Glomus claroideum Schenck & Smith
Glomus geosporum (Nicolson & Gerdemann) Walker
Scutellospora dipapillosa (Walker & Koske) Walker & Sanders
Scutellospora heterogama (Nicol. & Gerd.) Walker & Sanders
Acaulospora dilatata Morton

- b. Establishment and maintenance of an *in vivo* collection of the arbuscular mycorrhizal fungi

We have established and are maintaining in plant culture an arbuscular mycorrhizal fungi collection

<u>Specie:</u>	<u>From:</u>	<u>Maintained at:</u>
<i>Glomus mosseae</i>	Canary Islands	ICIA and IRTA
<i>G. mosseae</i>	England	ICIA and IRTA
<i>G. intraradices</i>	Barcelona	ICIA and IRTA
<i>G. aggregatum</i>	Barcelona	ICIA and IRTA
<i>G. clarum</i>	Brazil	ICIA
<i>G. etunicatum</i>	Barcelona	IRTA
<i>G. manihotis</i>	Colombia	ICIA and IRTA
<i>G. aggregatum</i>	Cuba (IES-4)	IRTA
<i>G. spurcum</i>	Cuba (IES-3)	IRTA
<i>G. mosseae</i>	Cuba (IES-8)	IRTA
<i>Glomus</i> spp.	Canary Islands	ICIA

- c. Supplying inocula to other participants involved in the project

We have supplied AMF inocula from Canary Island to:

- Annemie Elsen (KUL) : *G. mosseae*
- Stéphane Declerck (UCL-MBLA): *G. spurcum*, *G. aggregatum* (both from Cuba), *G. etunicatum* and *G. mosseae*

WP3: AMF formulation and inoculation procedures for micropropagated bananas and plantains

- a. Studies of the effect of mycorrhizae on growth of the micropropagated banana plantlets during the acclimatization phase in the nursery
 - Plants inoculated with *G. mosseae* (collection) and *G. mosseae*-Kollman (local isolate), showed a significative increase in all the development variables measured.
 - This increase was also significative in the foliar nutrient contents
- b. Determination the effect or two different AMF by studing the morphological and topological aspects of root system and changes in development on two commercial banana cultivars
 - Results after 9 months showed that plants inoculated with *G. intraradices*, particulary ‘Gruesa’ (Dwarf Cavendish) plants, were better developed, averaging 40% RMD rates
 - Conclusions of this trial confirm the long-term benefit of mycorrhizal application
- c. Determination and comparation the ability of different AMF isolates (mantained in collection) on micropropagated banana plantlets during the first stages of development in the nursery
 - Results after 2 months showed that each AMF had a particular behaviour
 - Plants inoculated with *G. aggregatum*, *G. mosseae*, *G. intraradices* and *Glomus* spp. (Cuba), showed the highest increasemente in development parameters, averaging 30% RMD rates
- d. Study the interaction between two beneficial microorganisms (AMF and PGPRs), on different commercial clones of *Musa*
 - Combined inoculation of AMF and PGPRs showed positive effects on banana development in all the cultivars studied, although the intensity of these effects varied among the cultivars
 - Since there are few reports on banana and it is known the high degree of especificity within microorganisms relations, some additional trials should be conducted
- e. Study the effect of alginate entrapped *in vitro* produced *Glomus* strains on the first stages development of micropropagated banana plantlets
 - The three AMF species entrapped in beads showed a good colonization ability, producing an increase in development and nutrition of banana
 - Banana plantlets showed a marked mycorrhizal dependency in all cases
 - Results showed that this new AMF inoculum can be taken into account as an effective and posible type of inoculum

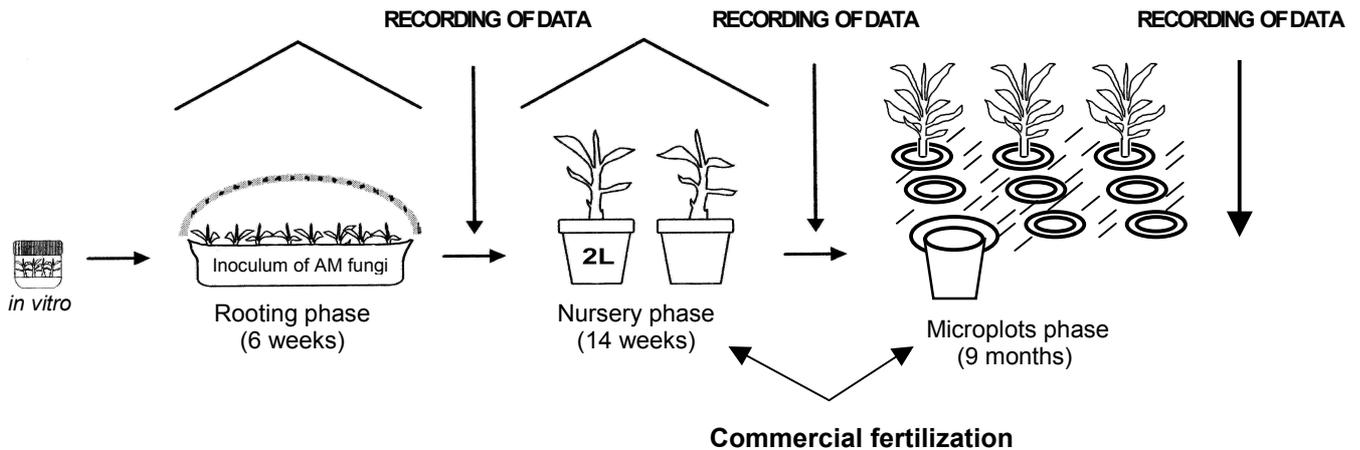
WP5: Interaction studies between mycorrhizal banana and plantains and their main root pathogens

- a. Evaluation the host suitability of four cultivars of local bananas against five isolates of lesion nematode *Pratylenchus goodeyi*
 - In all cultivars, local isolates were less aggressive than foreign ones
 - The four cultivars showed a similar susceptibility degree within each nematode local isolate
 - In the case of the two foreign nematode population, one cultivar ('Johnson') seemed to be less susceptible than the three others
- b. Study the evolution of the root-knot nematode population *Meloidogyne javanica* in the presence of *Glomus manihotis* in banana
 - *M. javanica* did not seem to affect mycorrhizal colonization
 - AMF seemed to reduce nematode symptoms in roots
 - Nematode reproduction rates inside the root were lower when the AMF is present
- c. Study the effect of the AMF *Glomus mosseae* on micropropagated banana infected with a high dose of *Fusarium oxysporum* f.sp. *cubense* (FOC)
 - FOC did not seem to affect mycorrhizal colonization
 - AMF seemed to have a beneficial effect on the infected plants
 - Fusarial symptoms appeared to be lower in plants with AMF, although no significant difference was observed
- d. Study the response of micropropagated banana cultured under microplots conditions in soil infected with lesion nematode *Pratylenchus goodeyi*
 - A marked effect of *Pratylenchus goodeyi* was observed, since damage was clear in plants with infection
 - AMF effect does not seem so evident, especially at the end of trial when AMF colonization appeared to decrease due to fertilization
- e. Study the combined effect of two beneficial microorganisms: AMF and PGPRs on micropropagated banana infected with the root-knot nematode *Meloidogyne javanica*
 - Each PGPR strain showed a particular behaviour against nematode (from weakly to strongly positive)
 - In the same way, each AMF-PGPR strain combination had a different effect on nematode infection
 - Consequently the microorganisms effect on banana development depended on the combination AMF-PGPR

4. Problems encountered

No problems were encountered during the time-course of the project.

5. Technology Implementation Plan



6. Publications and papers

6.1. Chapters in books

- **Azcón-Aguilar, C., Jaizme-Vega, M.C. and Calvet, C. 2001.** The contribution of arbuscular mycorrhizal fungi to the biological control of soilborne plant pathogens. *In: "Mycorrhiza technology: From Genes to Bioproducts.* Ed.; Hannes Schüepp. Life Science Editorial Department, Birkhäuser Verlag AG, Basel, Switzerland. pp:187-197.
- **Calvet, C., Estaún V., Camprubí, A. and Pinochet, J. 2002.** Interactions between arbuscular mycorrhizal fungi and non-symbiotic beneficial microbiota. *In: "Arbuscular mycorrhiza: Interactions in plants, rhizosphere and soils"*. Ed. Science Publishers Inc., EEUU. Pp: 29-45
- **Estaún , V., Camprubí, A., and Joner, E. 2002.** Selecting arbuscular mycorrhizal fungi for field application. *In: Mycorrhizal technology: : From Genes to Bioproducts.* Ed.; Hannes Schüepp. Life Science Editorial Department, Birkhäuser Verlag AG, Basel, Switzerland. pp:249-260.

6.2. Papers published

- **Calvet, C., Pinochet, J., Camprubí, A., Estaún, V. and Rodríguez-Kábana, R. 2001.** Evaluation of natural chemical compounds against root-lesion nematode and root-knot nematodes and side effects on the infectivity of arbuscular mycorrhizal fungi. *European Journal of Plant Pathology* 107:601-605
- **Camprubí, A., Calvet, C. and Estaún , V. 2000.** Micorrizas arbusculares en producción agrícola. *Horticultura*, 144:38-41.
- **Jaizme-Vega, M.C., Esquivel, M., Tenoury, P. y Rodríguez-Romero, A.S. 2002.** Efectos de la micorrización sobre el desarrollo de dos cultivares de platanera micropropagada. *Infomusa*. Vol 11(1): 25-28.
- **Jaizme-Vega, M.C., Declerck, S., Rodríguez-Romero, A.S. and Marín Hermoso, C. 2003.** Growth of micropropagated bananas colonized by root-organ culture produced arbuscular mycorrhizal fungi entrapped in Ca-alginate beads. *Plant and Soil* (in press)

6.3. Contribution in Congress

- **Jaizme-Vega, M.C., Tenoury, P. y Esquivel, M., 2000.** Follow-up of AM fungi on commercial banana cultivars in field conditions. Abstracts COST Action 838. Galician Institute for Agro-biological Research. Santiago de Compostela, Galicia, España, May 18-20, 2000.
- **Jaizme-Vega, M.C., Piñero, M.S. y Tenoury, P. 2000.** Efecto de la interacción de micorrizas y rizobacterias sobre el desarrollo de cuatro clones de *Musa* micropropagados. Resúmenes Reunión ACORBAT, S. Juan de Puerto Rico. 31 Julio-4 August 2000.
- **Rodríguez-Romero, A.S., Jaizme-Vega, M.C., y Tenoury, P. 2000.** Efecto de *Glomus manihotis* sobre la reproducción de *Meloidogyne javanica* en platanera. Resúmenes del X Congreso de la SEF. Valencia, España, October 3-6, 2000.
- **Jaizme-Vega, M.C., Declerck, S., Rodríguez-Romero, A.S. and Marín Hermoso, C. 2001.** Effect of *in vitro* monoxenically produced arbuscular mycorrhizal fungi on the first stages development of micropropagated banana plantlets. Abstracts COST 838 Action. Pruhonice, Czech Republic, September 26-29, 2001.
- **Rodríguez-Romero, A.S., Jaizme-Vega, M.C., and Barroso Núñez L.A. 2002** Effect of arbuscular mycorrhizal fungus *Glomus manihotis* on micropropagated banana cultured on soil naturally infested by lesion nematode under microplots conditions. 4th International Congress of Nematology. TenBel, Las Galletas, Tenerife, Canary Islands, Spain, June 8-13, 2002.
- **Jaizme-Vega, M.C., Rodríguez-Romero A.S. and Barroso Núñez L.A.** Evaluation of four commercial Arbuscular Mycorrhizal Fungi inocula on micropropagated banana on the first development stages. Abstract COST ACTION 838 Meeting AM research in Europe, Pisa, Italia, October 10-12, 2002.
- **Jaizme-Vega, M.C., A.S. Rodríguez Romero y J. Hernández Hernández.** Efecto protector de *Glomus mosseae* sobre platanera micropropagada afectada por el “Mal de Panamá” (FOC). XI Congreso de la Sociedad Española de Fitopatología, Almería, España, October 14-18, 2002.
- **Rodríguez Romero, A.S. y M.C. Jaizme-Vega.** Efecto de rizobacterias promotoras del crecimiento vegetal sobre la reproducción de *Meloidogyne javanica* en platanera. XI Congreso de la Sociedad Española de Fitopatología, Almería, España, October 14-18, 2002.

7. Workshops

- **Jaizme-Vega, M.C. 1998.** Application of Arbuscular Mycorrhizal Fungi in micropropagated banana. Organic and environmentally friendly banana production. Proceedings of a workshop held at EARTH, Guácimo, Costa Rica, 27-29 July 1998. Eds. Rosales, Tripon y Cerna
- **Jaizme-Vega, M.C. y Rodríguez-Romero, A.S. 2002.** Aplicación de micorrizas sobre el cultivo de platanera. Avances de la investigación en Canarias. Actividades del ICIA en Platanera. Febrero 2002. Eds. Fernández Galván y Hernández Delgado. Gobierno de Canarias
- **Jaizme-Vega, M.C. y Rodríguez-Romero, A.S. 2002.** Uso potencial de hongos micorrícicos para el control de patógenos de raíz. Inducción de resistencia y uso de tecnologías limpias para el manejo de plagas en plantas. Memorias del taller

internacional realizado en el CATIE, Turrialba, Costa Rica, 27-30 Agosto 2002. Eds. Riveros, Pocasangre y Rosales.

8. Master Thesis and PhD Tesis

- **Hernández Martín, B. 1999.** Estudio de la interacción de los hongos formadores de micorrizas arbusculares (MA) con *Pratylenchus goodeyi* sobre platanera micropropagada. CSCA, Universidad de La Laguna. Master Thesis.
- **Esquivel Delamo, M. 2000.** Aptitud y dependencia micorrícica de dos cultivares comerciales de platanera. CSCA, Universidad de La Laguna. Master Thesis.
- **Marín Hermoso, C. 2001.** Efecto de tres especies del hongo MA *Glomus* sp. producido axénicamente sobre el desarrollo de platanera micropropagada. CSCA, Universidad de La Laguna. Master Thesis.
- **Piñero Guerra, M. S. 2002.** Efecto de la interacción de micorrizas y rizobacterias sobre el desarrollo de cuatro clones de *Musa* micropropagada. María Sol. CSCA, Universidad de La Laguna. Master Thesis
- **Rodríguez Romero, A.S. 2003.** Alternativas biotecnológicas en cultivares de *Musa* frente a los principales patógenos de suelos en Canarias. Dpto. Biología Vegetal, Facultad de Farmacia. Universidad de La Laguna. PhD Thesis.

9. Exchanges of personal: training activities

- **Valérie Molinier from CIRAD:** visited at the IRTA (Cabrils, Barcelona) (July 1998)
- **Dr. A. Camprubí from IRTA:** visited at the UCL (Louvain-La-Neuve) Dr. S. Declerck's Department (October 1999)
- **PhD Student A.S. Rodríguez-Romero from ICIA:** visited at the CIRAD-AMIS (Montpellier) Dr. J.L. Sarah's Department (October 1999)
- **PhD Student A.S. Rodríguez-Romero from ICIA:** visited at the "Universitat de Girona" (Spain) Dr. E. Montesinos's Department (January-February 2001)

10. Conclusions

WP1: Establishment of a collection of Arbuscular Mycorrhizal Fungi (AMF)

Within this workpackage, seven AMF strains from the Canary Islands have been isolated and classified. An AMF collection containing isolates from tropical regions, has been also established by using plant culture. Our group has contributed to global objectives of this project, by supplying AMF inocula to other participants.

WP3: AMF formulation and inoculation procedures for micropropagated bananas and plantains

- Local or collection AMF isolates are able to improve plant development and nutrient uptake in micropropagated banana, during acclimatization phase in the nursery.
- Combined inoculation of AMF and “Plant Growth Promoting Rhizobacterias” (PGPRs) showed positive effects on development and nutrition of different commercial clones of *Musa*.
- The *in vitro* *Glomus* species entrapped in alginate beads showed a good colonization ability, and producing an increase in development and nutrition of banana. This new AMF inoculum can be taken into account as an effective and possible type of inoculum.

WP5: Interaction studies between mycorrhizal banana and plantains and their main root pathogens

- The nematode reproduction rates inside the root were lower when the AMF is present
- AMF have a beneficial effect on micropropagated banana infected with *Fusarium oxysporum* f.sp. *cubense* (FOC). Fusarial symptoms appeared to be lower in plants with AMF.
- When study the combines effect of two beneficial microorganisms (AMF and PGPRs) on micropropagated banana plant infected with the root-knot nematode, each PGPR strain showed a particular behaviour against nematode in the same way, and each AMF-PGPR combination had a different effect on nematode infection.