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PROJECT IP-06

Tropical Fruits, a Delicious Way to Improve Well-being

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

ANNUAL REPORT 2002 IP-6

Title: Tropical Fruits, A Delicious Way to Improve Well-being

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Within CIAT: Integrated Pest and Disease Management (PE-01); Land Use in America (PE-04); Rural Agroenterprises SN-1; Participatory Research SN-3; Soil, Water and Nutrient Management SW-2; Conservation and use of Genetic Resources SB-2.

Outside CIAT: National Programs: Colombia (CORPOICA, SENA, Corporación Colombia Internacional); University of Queensland (Australia); PROTA-University of Wageningen (Holland); ICRAF; GFAR; University of Florida

1. Budget:

Source	Amount (US$)	Proportion (%)
Unrestricted core	104,044	63
Core substitution	20,000**	10
Carry over from 2001	(8,000)*	(4)
Sub-total	**116,044**	**59**
Special projects	80,000**	41
Totals	**196,044**	**100**

* Consulting contracts completed during 2001 that were charged in 2002

** Estimates. These funds still have not been finally allocated at the time of writing.

[1] Many of the research and non-research staff of CIAT have been involved in the discussions that have been crytallised in the strategies and operational plans of the Tropical Fruits Program. They are not listed here, but their input is hereby acknowledged and greatly appreciated.

2. *Highlights in 2002*

The Centre's efforts in tropical fruit research and development until the latter half of 2001 comprised a series of special projects designed principally to resolve problems identified by the Ministry of Agriculture and Rural Development of Colombia (MADR). In 2001 an independent consultant was hired to establish the basis for implementing an international Tropical Fruits Programme. Towards the end of 2001 the Board of Trustees approved the establishment of a Tropical Fruits Programme and indicated that the centre should structure such a programme.

A dual strategy is being used to establish the programme. The limited resources of basically one internationally recruited staff member (75%) and support are being used to (i) develop the overall strategies of the programme so as to attract special project financing and (ii) to initiate research, based on the long term strategies, that will help attract funds and (iii) continue with research to resolve problems identified by the MADR.

In this report we present the programme strategy, programming of future activities and reports of research activities. Several of the research activities are presented in research paper format.

2.1. Programme Strategy.

In the past the international centres' commodity programs set out from the beginning to become the dominant research and development organization in the commodity concerned and then transferred the knowledge gained to its partners in national programs, which tacitly accepted the dominant position of the IARC program. In the case of the myriad tropical fruits it is evident that the national programs will have to play the dominant role for each specific fruit crop and the IARCs will provide support to them to achieve their objectives.

2.1.1. Tropical Fruits and Rural Development.

There are a vast number of different tropical fruits originating mainly from SE Asia, Africa and the Americas but only a small number of species or genera dominate the production scene. Citrus, bananas and plantains, mangoes and pineapples provide 89% of the total production. The total production of tropical fruits is dynamic with production doubling approximately every 20 years. Ninety eight percent of this production is in the developing countries.

The tropical fruit crops (excluding citrus and bananas) are mainly consumed as fresh fruit in the developing countries where they are produced. Over the last ten years in Latin America and SE Asia there has been a remarkable increase in the consumption of processed fruits. These changes in food consumption habits reflect changes in the class of the product being consumed rather than one species substituting for another. Although it is estimated that 96% of the tropical fruit production in the developing countries is consumed locally, they are increasingly looking to exports of these fruits as a means to increase rural incomes and foreign exchange earnings. Simultaneously the developed countries are consuming more non-traditional fruits from the developing countries. Furthermore, there is an increasing trade in tropical fruit products between developing countries themselves.

In 1995 the total value of tropical fruit exports (excluding bananas and citrus) was estimated at 1.5 million t with a value of US$1.3 billion. The average estimated value of the exported produce is about US$9,000 per ha. Whilst not all of the value added is in the rural areas, there is no doubt that a significant portion can be maintained in the rural sector. The total value of produce for the local market tends to be much less; nevertheless with an average figure that is in the region of US$3,000 per ha it is greater than most alternatives.

Fruit consumption is increasing as whole, and the rates of increase of production of most individual species are high. The urbanisation of society and interest in a healthy diet has opened up an expansive market for fruits both within the developing and developed countries. Although the aggregate demand for exotic fruits may be large, the demand for individual species or products may be quite limited and rapidly saturated.

The production is concentrated in a small number of countries, with exports of specific fruits largely from only one or two countries. Thus in tropical fruits the benefits of development of a particular species tend to be localised. However due to the high value of the produce and the intense labour requirements they can be extremely important in the local community.

The tropical fruits offer the opportunity to increase incomes in the rural areas either through increasing farmers' incomes or employment opportunities to the landless. A producer of tropical fruits may achieve a reasonable standard of living from a holding of limited area. In addition, development of fruit based agro industry normally leads to accelerated development of the region. Furthermore, most tropical fruit crops are perennials making them extremely attractive in hilly ecosystems where continuous ground cover and minimum tillage are a prerequisite for sustainable management systems. In addition with a high income per unit land area they are unlikely to be grown on vast areas, and hence there is little danger that expansion of production will lead to clearing of large areas of land and major disturbances to the ecosystem. The association of fruits with a healthy way of life by the consumer is reflected in production practices. Consumers of healthy products such as tropical fruits will insist that they have not been doused with massive quantities of noxious chemicals. Consequently, the cultural practices will tend to be environmentally benign in terms of use of agro chemicals. Thus tropical fruits offer the opportunity to alleviate poverty in rural areas whilst at the same time providing a healthy and pleasant environment.

2.1.1.1. Mission and Vision.

The tropical fruits are seen as an attractive vehicle for sustainable rural development in many tropical countries with the vision of *"Vibrant rural communities that provide a pleasant life and healthy environment for their constituents and their children principally through the wealth generated by producing, processing and marketing tropical fruits."*

Within this context the mission of the CIAT tropical fruits programme is *"to use science, technology and modern information technology to provide information and support to partners in the public and private sector that promote production, processing and marketing of tropical fruits by rural communities which leads to increased wealth and improved welfare for present and future generations in the countryside."*

2.1.2. Local Needs and Global Strategies

The development of any one tropical fruit into a successful vehicle for increasing incomes of a substantial number of people is dependent on an expansive market. The local markets for individual tropical fruits are frequently insufficient to justify large investments in their development. The policy framework in most developing countries is to develop programs for export, whilst recognizing that the availability of tropical fruits and fruit products in the local markets will also increase.

Certain tropical fruit species, particularly those that can be considered as well-developed fruit crops for export (eg bananas from Central and South America, citrus products from Brazil) are already well supported by functioning research and development agencies. No role is seen for the CIGIAR system to further support them. On the other hand, there are a vast number of crops of local importance for internal consumption and potential export crops that have been bypassed by modern technology. The CGIAR should direct its efforts to these crops.

The efforts of the CGIAR and CIAT in particular in this area will only be a part of a continuum of activities carried out by several organisations from the public and private sector. Taking a pan-tropical stance, the potential for generating income for a large number of people in various regions and ecologies depends on satisfying the demand for a variety of different fruits, flavours and health products. In this sense the pan-tropical view, which must be taken by an international centre, diverges from that of a national research centre or private enterprise that will probably try to carve out its own particular local niche and establish itself as the leader in a small number of fruits, flavours and health foods. Consequently, international centres must find the means of providing broad support to the national programs on tropical fruits in general, whereas the local organisations should concentrate on specific crops.

2.2. Research Programming and Results

The activities of broad based support given by CIAT is directed to four principal areas:

- Defining what crops can be grown where
- Market intelligence
- Generic research to open new opportunities in a number of crops
- Rural agro enterprises.

In addition the Centre provides the service of specific problem solving contract research in areas where it has a particular competence.

2.2.1. What can be grown where: Homologue™ and CropIdent™

In agriculture one of the foremost questions is "What can be grown in a specific site". Farmers wish to know what will grow well on their farm or a particular field on their farm. Answering this question provides the initial basis for analyzing and then defining the best possible business options in agriculture. Farmers, agri-business, food processors, supermarkets, development agencies, local planning organizations, public agricultural organizations and credit organizations are all likely to ask this question on repeated occasions. In the case of the major commodity crops information is available on those species and varieties that are likely to perform well under specific conditions. In the case of the myriad crops considered to be of minor importance on a global scale, but of great importance to rural communities and local economies, reliable information on what could be successfully grown is not readily available and is largely based on local or expert knowledge and access to literature organised on a species by species basis. The local and expert knowledge tends to be restricted to those species or crops already grown in the specific region or at least on the same continent.

The geographical constraint on knowledge is of particular concern in the light of the consistently superior performance of non-native species in commercial agriculture throughout the world. This is true of tropical fruits at present and is likely to continue to be so, thus, the most promising potential new crops for local consumption or for export will come from regions distant from the potential producer/exporter. Frequently the potential producers of a specific crop in one continent have no idea of the existence of that crop or how to obtain germplasm and grow it. In addition, at present there is little or no incentive for one country to collect and develop germplasm and the accompanying technology knowing that its efforts will primarily benefit other regions of the world.

In order to identify species likely to perform well under a given set of conditions an enquirer has to sift through large amounts of information on species that are not relevant to his individual needs. The advent of computerised Geographical Information Systems (GIS) opens the way to managing the large amount of data required to match particular crops or species to the particular growing conditions of specific sites. We have developed the conceptual basis for the development of two interlinked systems, CropIdent™ and Homologue™, that will enable users to determine what crops or varieties are likely to grow well in a given site.

A combination of expert opinion and local knowledge on the requirements of particular species, combined with the identification of a small number of specific sites where the crop or species is known to grow well in the CropIdent™ system will be fed into Homologue™ which will identify other similar areas or "homologues" where the crop is likely to be successful. An important advantage of this approach is that there is no requirement for specific crop parameters related to such individual factors as for example critical temperatures for pollination: the fact that a crop grows and performs well in a known site which is adequately described is sufficient information.

The simple model we propose depends on two basic premises. Firstly that qualitative information on performance of a crop or species in characterised sites combined with expert opinion and local indigenous knowledge can be used to determine the Site Characteristics for Good Crop Performance (SCGCP) of

particular crops. Secondly once the SCGCP have been determined these can be used to either identify other sites where the crop could be successfully grown or to indicate the likelihood of that crop being successfully grown in a predetermined target site with known characteristics.

The proposed system will be built on our experience at CIAT with the development of MARKSIM™ , which manages large climatic databases and FLORAMAP™ , which uses information on the known distribution of species to determine the probability of finding that species in another site. In addition experience already gained in linking expert knowledge with GIS in forage species in Central America will be used to develop the SCGCP tables, which are the basis for development of CropIdent™.

The site characteristics will be managed in a hierarchical framework with climatic factors being the highest level and soil, topography etc. being managed at a lower level. This division is based on the observation that the spatial variation of climate and soils is generally such that soil and topographical features form sub-sets within any one climate set. A consequence of this is that existing date bases can be used to determine, with reasonable accuracy, the Site Climatic Characteristics (SCC) of any geo-referenced point, whilst only the predominant soil and topographical characteristics of the region can be inferred. However, plants do not respond to the predominant conditions of a region but rather to the actual conditions at the point or site where they grow. Hence for each site a Rapid Soil Appraisal (RSA) will be needed to support the site climate data. Expert or local knowledge will then be used to identify sites where the crop grows well and to describe the soil conditions with or without visits.

The question normally posed will be what can I grow in a particular area, with a homogeneous climate but with varying soil conditions. The climate of an area can be obtained from the database by providing the geo reference of the area. The existing soil database will then be used to provide a rough estimate of the potential area with suitable soil conditions or a more precise estimate if Rapid Soil Appraisal data exist. The process software will then compare the climate conditions with climatic SCGCP for each crop for which SCGCP tables exist. This process will pre-select crops suited to the climatic conditions in the zone and will then provide a suitability index for each of them in each of the specified soil conditions.

Once such the system is developed it will have multiple uses: Farmers, farmer groups or other agencies would be able to identify potential species or crops for their particular conditions; farmers, technologists and planners interested in obtaining technology would be able to accurately identify the most promising sites or areas to visit; agronomists would be able to identify sites where particular genotypes and crop management technologies are likely to function effectively; farmers, planners and entrepreneurs would be able to identify areas in which the present climate is similar to the future climate in other areas and predict which existing crops and technologies are likely to be suitable for changing site conditions related to climate change.

2.2.2. Market Intelligence

The recommended approach for developing particular fruit crops is via a two-pronged attack. On the one hand production experts look at which crops show potential in production terms in a given region, whilst at the same time marketing specialists analyse the market demand and requirements for different classes of products. The CIGIAR system can provide much of the general information required by national agencies and local organizations on potential production of crop species under particular conditions. The national programs can then analyse this information from their own particular perspective, and couple it with general and specific information on markets so as to make coherent investment decisions.

The role of international centres should not be that of looking at the day-to-day fluctuations in prices nor to analyse in depth the demand for a specific fruit or product. Rather the market intelligence should be restricted to analysing expected long-term future trends in the demand for classes of products. This information would then be used by local organizations to develop specific products based on individual or combinations of a limited number of fruit species.

The market intelligence will be obtained by first bringing together experts in the field on a periodic basis in workshops and second, when the groups of experts deem that it would be advisable, to commission specific studies on classes of products that are considered to have potential.

2.2.3. Rural Agricultural Enterprises

In order to fulfill its mission, the Tropical Fruit Crops Program must achieve equitable distribution of benefits along the production chain. With the same final product, project design can greatly influence the distribution of benefits. With its long experience in the integrated production, processing and marketing of cassava and more recently the Rural Agro-Enterprise Development and CODESAN, CIAT will bring its experience to bear on the design and implementation of projects with the specific goal of ensuring equitable distribution of benefits.

The focus of the Tropical Fruits Programme is to provide farmers, groups of farmers and diverse development agencies information that broadens the scope of their options to link the rural community to modern markets. On the other hand Rural Ago Enterprise Development specializes in the identification of groups interested in linking themselves to the market economy and helping them to identify and analyse different business options. Tropical fruits are amongst the best options for linking the rural sector to the modern economy. Within the context of rural development it is evident that Rural Agro Enterprise Development plays a role at a higher hierarchical level than Tropical Fruits. Rural Agro Enterprise Development first identifies groups interested in establishing agri-businesses and then if fruits are seen as a promising option the Tropical Fruits Program enters to provide support. Thus Rural Agro Entrprise Development takes the leadership in identifying and fomenting projects that link the rural population to the modern markets. At the same time an exciting aspect for the future development of rural agro-enterprises is the possibility of matching different tropical fruit species and products to different environmental conditions: it becomes possible to select a target population or region for rural business development and then select an appropriate fruit or fruit product for their specific conditions.

For more details of the progress in rural agri business development the reader is referred to the section in this report on Rural Agro Enterprise Development.

2.2.4. Generic Research on Common Problems

Much of the research to improve the production chain of any particular fruit crop will be highly specific to that fruit crop. These highly specific research areas will normally have to be covered by the local centres of excellence or consortia that they establish. Nevertheless we have identified several research themes that are directly relevant to many different fruit crops, and CIAT will explore the possibility of developing activities related to these issues.

2.2.4.1. Ripening and Post harvest deterioration.

Control of maturity and post harvest deterioration are major concerns in the marketing of almost all fruit species. In the case of the climacteric fruits the basic metabolism underlying ripening is essentially the same for all the different crops. In the non-climacteric crops it is probable that many of the processes associated with post harvest deterioration, such as softening and degradation of the cell wall, are common to a wide range of species. It is proposed that CIAT should explore new opportunities for control of ripening and post harvest deterioration. As CIAT, and the IARCs in general, are weak in the area of post harvest deterioration, we shall establish strategic alliances with agencies such as CIRAD or Universities that have expertise in these areas.

2.2.4.2. Flowering.

In order to produce any fruit the plant must flower. Synchronisation and control of flowering, and hence fruiting, is a fundamental part of all organized fruit production systems. The bulk of the knowledge on the underlying principles of control of flowering is based on research on temperate species. Relatively little research has been carried out on flower induction and control of tropical species under tropical conditions. At the same time recent discoveries in plant science indicate that similar mechanisms under genetic control the flowering of a wide range of species. We suggest that a better understanding of the flowering processes in tropical species would facilitate development of practical techniques for flowering control in the field.

We have been developing propagation techniques for Lulo (*Solanum quitoense)* and Sour sop[2] (*Annona muricata)* and have observed at the field level that some of the *in vitro* techniques that we have used to take buds from mature stems produce plants that are early flowering.

2.2.4.3. Non crop specific diseases and pests.

Certain diseases and pests attack a wide range of different crop species. In the case of the fruit crops a small number of species of fruit flies damage the fruits of many species and complicate importation of many fruits for quarantine reasons. CIAT proposes to explore the possibility of studying fruit flies with a view to developing improved control measures and to facilitate the exportation of fresh fruit products. Preliminary contacts have been made with various Colombian entities, Aphis, ICIPE and the University of Florida, which have all shown interest in working on a joint program.

2.2.4.4. Propagation

Improved propagation techniques for tropical fruits offer multiple opportunities to improve production. Farmers frequently are unable to obtain seed or propagules of the varieties they desire, and in addition the quality of the planting material is often of low physiological and sanitary quality.

Many fruit species are open pollinated and when farmers sow seeds the resulting plants highly variable. This is both a problem and an opportunity. The problem for the farmer is that he is not sure of what he's planting, but this also provides the opportunity to select the best trees and use them as planting material. A major limitation to this type of farmer selection is the effective rapid clonal propagation of woody material.

In a joint project with Corporación Biotec we have used sour sop (*Annona muricata)* as a model plant to develop a system of rapid clonal propagation based on micro grafts of selected scion material onto stocks. Initially a local expert selected the scion material.

The selected material has been successfully propagated and planted at CIAT, Palmira and in various commercial plantations. Up to present the performance of the selected material has been excellent with prolific fruiting and high levels of production.

In the future we envisage scaling up the technique so that farmers in collaboration with nurseries can select materials that they find particularly attractive, propagate that material and plant it. This is an interesting example of how new technology developed at the laboratory level can help farmers carry out their own selection of improved cultivars or clones.

Work on regeneration of lulo done in another context (see below) also opens up the possibility of rapidly propagating clonal lulos for commercial production. Regenerated lulo planted in preliminary small scale trials in the Western Cordillera of the Andes at about 1800 m altitude have developed normally and produce abundant flowers and fruits.

2.2.5. Contract Research and Development.

A national organization identifies the problem to be solved or new opportunity in tropical fruits. It then decides if it has the in house capability to execute the task or if it would be preferable to collaborate with another organization. In the latter case the most desirable option maybe an international centre such as CIAT, in which case the national organization will contact CIAT and enter into a contract to carry out the research or development project in question. Most research of this type up to the present has been with the Ministry of Agriculture and Rural Development of Colombia.

Reports in these areas are presented in research paper format and are attached to this document.

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[2] This work is in conjunction with Corporación Biotec.

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Development of methodologies for in vitro multiplication, plant regeneration, and genetic transformation of naranjilla (lulo)

V. Segovia, Z. Lentini

Introduction

A large number of fruits of Andean origin have great potential to become premium products for local and export markets with a high economic return for the farmers. Naranjilla (Solanum quitoense) is among these fruits. This species is native from Colombia and Ecuador, and it is normally cultivated between 700 and 2000 meters above sea level. Some of the main attributes of this fruit includes its high level of vitamin C, and the sub-shrubby perennial growth amenable for cultivation in hillsides and inter-cropping, aiding soil conservation practices. Recently in Colombia, naranjilla changed from being a fruit of local fresh consumption to become an important industrial fruit for juice and yogurt products, increasing its market value. A major constraint for the rapid adoption of naranjilla by the local farmers is the limited availability of elite germplasm free of pathogens. The high level of trait segregation restrains its multiplication through seeds. Rapid multiplication of quality planting materials is of paramount importance. One of the main objectives of this project is to develop a protocol for in vitro propagation of naranjilla with application for conservation and rapid multiplication of clones free of pathogens. The expected results include the mass multiplication of elite clones that then can be distributed to farmers. Since breeding for this species is almost non-existing, paralelly to the in vitro propagation effort, it will be important to develop plant regeneration and transformation systems to aid the development of germplasm. Last year it was reported the advancement in establishing a system for maintenance of the in vitro germplasm collection, and the progress made identifying factors to increase the plant regeneration efficiency from elite naranjilla materials. This year it is presented the development of an efficient plant regeneration system, the evaluation of regenerated plants in the field and the comparison of the growth and development with in vitro propagated plants. The establishment of a protocol for introducing new material from plants growing in soil is also presented.

Materials and Methods

High quality and elite clones provided by the Andean Fruit Center (Centro Frutícola Andino – CEFA) were used. This collection includes naranjilla with or without thorns commonly grown by farmers. The effect of the in vitro propagation medium on the efficiency of plant regeneration was evaluated. Different propagation medium were tested using foam plugs to determine the interaction of the medium composition and free gas exchange. A protocol to readily incorporate material in vitro from the greenhouse or the field was optimized. A small scale field trial was conducted at 1700 m over sea level and a mean temperature of 22C was conducted to compare the growth and development of regenerated plants respect to in vitro propagated clones.

Results and Discussion

A randomized block design of four replicates each of 15 experimental units was used to determine the best medium composition and explant to induce a direct plant regeneration in naranjilla. A non-parametric chi-square analysis indicated that petioles showed showed from 3 to 9 times more more plant regeneration that the corresponding leaves from thorny and non-thorny clones respectively (Figure 1A). A significant higher response was also noted on medium originally develop for plant regeneration of tomato (Ultzen et al, 1995), consisting on MS salts, B5 vitamins, supplemented with sucrose 10 g/l, glucose 10 g/l, gelrite 1.5 g/l, zeatine 2 mg/l and IAA 0.02 mg/l (Figure 1A). On this medium petioles from thorny genotypes showed twice increase in plant regeneration respect to a medium reported for naranjila (Hendrix et al., 1987) composed by MS salts and vitamins and supplemented with sucrose 30 g/l, agar 7 g/l, IAA 0.01 mg/l, kinetin 5 mg/l, or with a modification consisting on gelrite 2 g/l and BAP 2 mg/l (modification suggested by Dr Richard Litz, Univeristy of Florida, laboratory which Hendrix work was conducted)(Figure 1A). Non-thorny genotypes did not regenerate any plant on medium developed by Hendrix (Figure 1A). The efficiency in response was highly affected by the medium composition on which the petiole donor plant were grown. Petioles of plants grown on medium A (MS basal salts and vitamins, and supplemented with calcium pantothenic acid 2.5 mg/l and gelrite 3.5 g/l) showed about 20% more plant regeneration respect to petioles from plant grown on medium ½ MS(½ MS basal salts supplemented with ANA 0.02 mg/l, BAP 0.04 mg/l, and GA_3 0.05 mg/l) (Figure 1B). Although plants developed better on media CEFA (MS salts with Tiamina 0.4 mg/L and Inositol 100 mg/L, gelrite 2 g/L y BAP 0.5 mg/L) or Corpoica (MS salts with Tiamina 0.4 mg/L and Inositol 100 mg/L, gelrite 2 g/L y BAP 0.5 mg/L) when foam plugs rather than plastic caps were used to seal the test tubes, the best development is obtained in medium A.

The re-establishment of an in vitro collection derived from greenhouse or field grown plants is under progress by using apical meristems instead of axillary's buds as the starting materials. Shoots derived from axillary's buds showed high levels of contamination. The current protocol used consist of sterilization with 70% ethanol for 1 min, followed a wash with water and then immersion in 1% sodium hipochlorite for 15 min. Then three washes with sterile bi-distil/ de-ionized sterile water. Apical meristems are cultured on medium A supplemented NAA 0.02 mg/L, BAP 0.04 mg/L and GA3 0.05 mg/L.

Field comparison of regenerated plants respect to in vitro propagated plants, indicate that naranjilla plant growth and development appears not to be affected by the organogenesis process (Figure 2). Plant height, leaf morphology, and plant type as well as days to flower formation, anthesis, and fruit development were similar in the two types of plants (Figure 4). In both cases, plants flowered 45 days after transplanting in the field (about 90 days after transfer from in vitro conditions to the soil in the greenhouse prior to the field), and fruit formation started at 65-70 days (Figure 3). These results indicate that in vitro derived naranjilla plants developed significantly earlier respect to standard crop, which flower at 150 days and fructify at 270 days.

Future plans

- Evaluate other factors affecting plant regeneration, especially those associated with ethylene effect(s)
- Develop a genetic transformation protocol
- Evaluate a medium scale field trial to complete evaluation of yield in regenerated plants and compare the growth and development with in vitro propagated
- Collaborate with farmers to compare the production of in vitro derived plants with those from standard crop conditions

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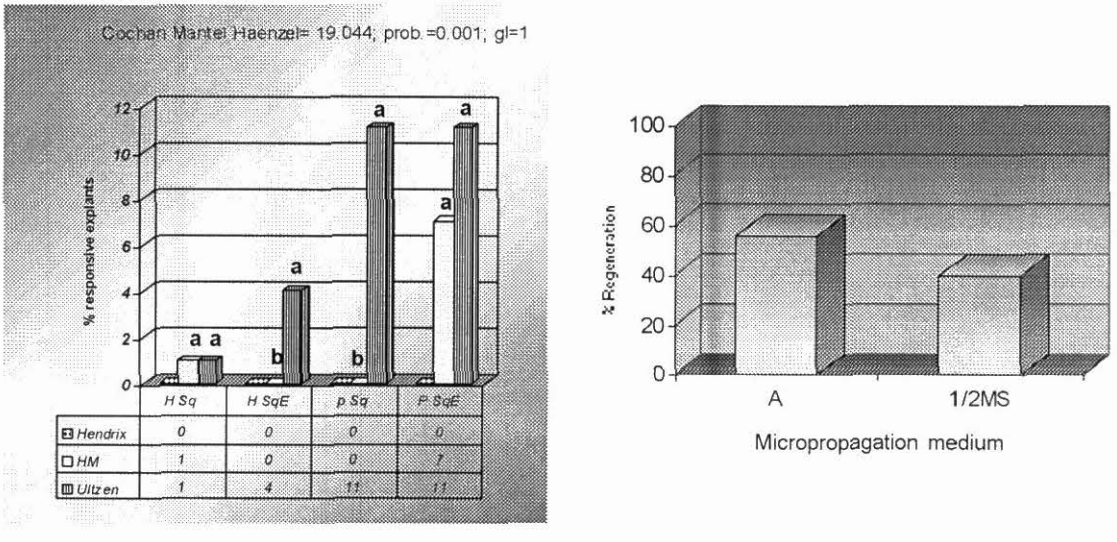

Figure 1. (A) Plant regeneration from petiole or leaf explants of genotypes with or without thorns using medium previously develop for tomato or reported by Hendrix for naranjilla. (B) Plant regeneration of petioles derived from plants micro propagated on medium A or 1/2MS.

Figura 2. Plant development in the field. Left, whole plant. Center , flower at anthesis. Right, fruits at 90 days.

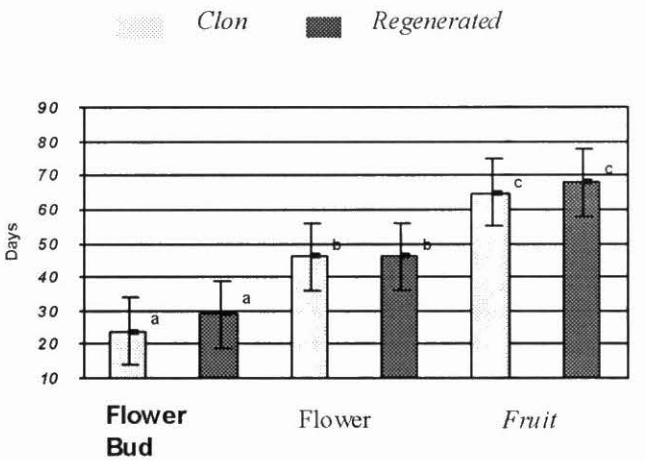

Figure 3. Development of field grown plants

Development of methodologies for *in vitro* multiplication, plant regeneration, and genetic transformation of naranjilla (lulo)

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A large number of fruits of Andean origin have great potential to become premium products for local and export markets with a high economic return for the farmers. Naranjilla (*Solanum quitoense*) is among these fruits. This species is native from Colombia and Ecuador, and it is normally cultivated between 700 and 2000 meters above sea level. Some of the main attributes of this fruit includes its high level of vitamin C, and the sub-shrubby perennial growth amenable for cultivation in hillsides and inter-cropping, aiding soil conservation practices. Recently in Colombia, naranjilla changed from being a fruit of local fresh consumption to become an important industrial fruit for juice and yogurt products, increasing its market value. A major constraint for the rapid adoption of naranjilla by the local farmers is the limited availability of elite germplasm free of pathogens. The high level of trait segregation restrains its multiplication through seeds. Rapid multiplication of quality planting materials is of paramount importance. One of the main objectives of this project is to develop a protocol for *in vitro* propagation of naranjilla with application for conservation and rapid multiplication of clones free of pathogens. The expected results include the mass multiplication of elite clones that then can be distributed to farmers. Since breeding for this species is almost non-existing, paralelly to the *in vitro* propagation effort, it will be important to develop plant regeneration and transformation systems to aid the development of germplasm. Last year it was reported preliminary results on developing a plant regeneration protocol. This year it is presented the advancement in establishing a system for maintenance of the *in vitro* germplasm collection, and the progress made identifying factors to increase the plant regeneration efficiency from elite naranjilla materials.

Materials and Methods

High quality and elite clones provided by the Andean Fruit Center (Centro Frutícola Andino – CEFA) and Corpoica La Selva are used. This collection includes naranjilla with or without thorns commonly grown by farmers. Various media were tested for *in vitro* maintenance of the clones. Statistical experimental design was used to determine the optimal plant tissue and medium for an increase efficiency in plant regeneration. Regenerated plants were taken to the greenhouse and the field to evaluate plant growth and development to maturity. Plants initially grown *in vitro* and then in soil, were taken back to the *in vitro* system to establish a protocol for renewing the *in vitro* collection.

Results and Discussion

In Vitro Propagation

Last year it was reported that plant develops healthier and faster *in vitro* when cultured onto ½ MS medium supplemented with ANA 0.02 mg/l, BAP 0.04 mg/l, and GA_3 0.05 mg/l, and agar 4.5 g/l, in contrast to the regular micro-propagation medium used by CEFA or Corpoica La Selva (SB2 Annual Report 2000). However, after 10 months of sub-culturing

naranjilla *in vitro* plants in this medium, most of the plants showed white stripes on leaves, thick stems, and slow development. Reason why other media were tested again. For this purpose, it was evaluated media commonly used for long term maintenance of *in vitro* germplasm of other *Solaneace* species. Of the medium tested, a medium containing MS basal salts and vitamins, and supplemented with calcium pantothenic acid 2.5 mg/l and gelrite 3.5 g/l (Hussey and Stacey, 1981) used for potato, also showed to be the optimal for naranjilla (medium A). On this medium, *in vitro* shoots start showing root proliferation two weeks earlier respect to medium ½ MS medium. Fully expanded new leaves were present at one month after subculture on medium A, whereas it took about 6 weeks on ½ MS medium (Figure 1). Moreover, new leaves developed on medium A were completely green although the starting materials had leaves with white stripes. The number of leaves with white stripes increased as plants were sub-cultured on ½ MS medium. By sub-culturing plants on medium A contained in jars rather than in test tubes, it is possible to obtain a large number of explants to conduct series of experiments for optimizing plant regeneration with various replicates every month. Medium A also seems to be a more appropriate for a long term in vitro maintenance of the germplasm collection since plants are healthier, develop faster, and differentiate normal green looking leaves.

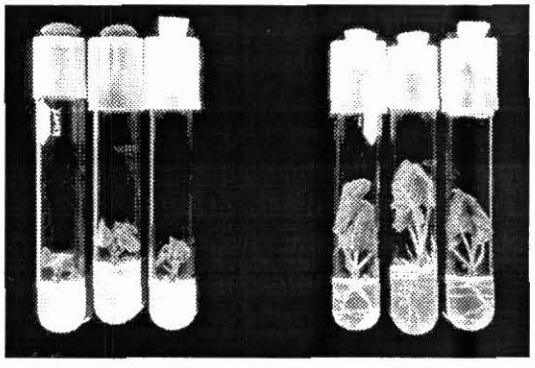

Figure 1. In vitro development of naranjilla plants in ½ MS medium (left) and medium A (right) one mothe after subculture

Plant Regeneration

A randomized block design of four replicates each of 15 experimental units was used to determine the best medium composition and explant to induce a direct plant regeneration in naranjilla. A non-parametric chi-square analysis indicated that petioles from the first and second node showed 17 times and about four times more plant regeneration that the corresponding leaves from thorny and non-thorny clones respectively. (Figure 2 and 3). A significant higher response was also noted on medium originally develop for plant regeneration on tomato (Ultzen et al, 1995), consisting on MS salts, B5 vitamins, supplemented with sucrose 10 g/l, glucose 10 g/l, gelrite 1.5 g/l, zeatine 2 mg/l and IAA 0.02 mg/l (Figure 2A). On this medium petioles from thorny genotypes showed three fold increase in plant regeneration respect to a medium reported for naranjila (Hendrix et al., 1987) composed by MS salts and vitamins and supplemented with sucrose 30 g/l, agar 7 g/l, IAA 0.01 mg/l, kinetin 5 mg/l, or with a modification consisting on gelrite 2

g/l and BAP 2 mg/l (modification suggessted by Dr Richard Litz, Univeristy of Florida, laboratory which Hendrix work was conducted)(Figure 2B). Non-thorny genotypes did not regenerated any plant on medium developed by Hendrix (Figure 2B).

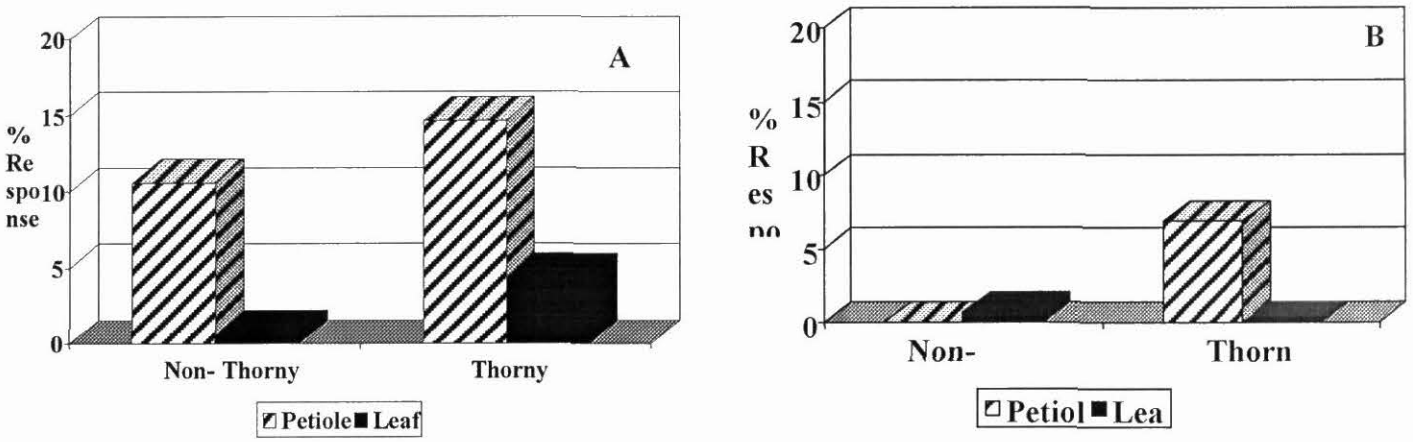

Figure 2.- Plant regeneration from petiole or leaf explants of genotypes with or without thorns using medium previously develop for tomato (A) or reported by Hendrix for naranjilla (B).

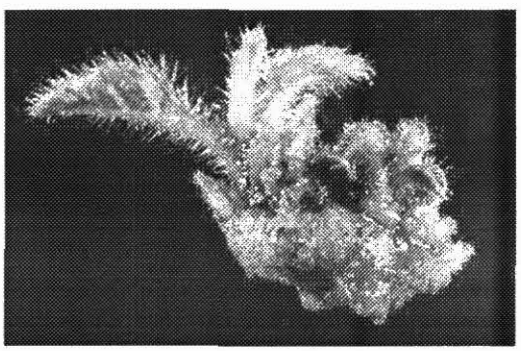

Figure 3.- Multiple shoot formation from petiole of naranjilla

Plant Evaluations in the Greenhouse and the Field

A methodology was establish to transfer *in vitro* material (from the *in vitro* germplasm collection and regenerated plants) to the greenhouse and there after, to the field. The first regenerated plants evaluated shown a normal plant growth and development to maturity in the greenhouse and in the field. Fruit formation is being evaluated in the field only, since temperatures in the greenhouse at CIAT headquarters is to high for naranjilla fructification. The field selected is located at 1,700 m.s.n.m. and with a mean temperature of 22 C, ideal for induction of fruit formation of naranjilla. The field plot is located in a farm (La Casona) at Dapa about 20 min from Cali, where there is a naranjilla production by farmers.

Future plans

- Establish a cyclic culture from greenhouse to *in vitro*, to completely renew the existing germplasm *in vitro* collection and propagate it in medium A
- Evaluate other factors affecting plant regeneration response to increase it at about 50%
- Develop a genetic transformation protocol
- Complete evaluation of regenerated plants in the field and compare the growth and development with *in vitro* propagated and seed derived materials

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106194

CONSERVACIÓN Y TRANSFORMACIÓN GENETICA DE LULO (*Solanum quitoense*) Y TOMATE DE ARBOL (*Cyphomandra betacea*)

Zaida Lentini

Introducción

Un gran número de frutas de origen Andino tienen gran potencial de convertirse en productos *premium* de consumo Nacional y de exportación con un alto beneficio económico para los agricultores, al tiempo que pueden ser utilizados para la conservación y regeneración del ecosistema de laderas. Entre estas frutas encontramos el lulo (*Solanum quitoense*), originario de Colombia y Ecuador, el cual normalmente se cultiva entre 700 y 2000 m.s.n.m., al igual que el tomate de árbol (*Cyphomandra betacea*). Un indicador de los posibles cambios que el mercado de frutas puede propiciar se registró en los últimos años con el lulo, el cual pasó de ser una fruta de consumo fresco local para entrar a la industria de jugos, lo que incrementó su valor agregado abriendo nuevos mercados. Sin embargo, al igual que la mayoría de las frutas tropicales, estas especies tienen limitantes importantes que restringen su cultivo, como son la susceptibilidad a enfermedades y pestes, problemas de deterioro en la poscosecha, heterogeneidad en la calidad de la fruta, y una disponibilidad limitada del material elite libre de patógenos. El control de estos factores incrementan los costos de producción. El fitomejoramiento genético es una alternativa clave para el desarrollo de materiales mejorados con resistencia a pestes y enfermedades, y de calidad superior. La práctica cotidiana del control químico para pestes y enfermedades, además de aumentar los costos de producción, es una fuente de contaminación que afecta no solo al ambiente sino a la salud. En el caso de frutales leñosos sin embargo, el período de fitomejoramiento es substancialmente mayor respecto a cultivos anuales, lo que dificulta el desarrollo de nuevas variedades. Esta limitante de tiempo puede ser asistida complementando el fitomejoramiento convencional con la micropropagación masiva de materiales elites libres de patógenos, y la ingeniería genética, que es una técnica que permite insertar genes directamente en el genoma de la planta obviando el proceso de cruzamiento sexual. Esta técnica además posibilita el uso de genes presentes en otras especies, incluyendo el acervo silvestre relacionado con la especie cultivada cuyo uso generalmente es restringido debido a barreras de incompatibilidad inter-específica. Los avances biotecnológicos de las dos últimas décadas principalmente se ha realizado con especies anuales de zonas templadas. La inserción de la Biotecnología en estas especies esta permitiendo la aplicación de estas técnicas en el desarrollo expedito de variedades comerciales, incrementando su competitividad en el mercado Internacional. En contraste, las especies de frutas tropicales han sido objeto de una atención muy limitada. Es claro que para incrementar el valor agregado de las frutas tropicales bajo el actual régimen de globalización y competitividad, se requiere el desarrollo, la adaptación y optimización de estas técnicas para agilizar el desarrollo de materiales mejorados. La tecnología desarrollada no solamente estaría apoyando al desarrollo de materiales mejorados, sino que además en sí misma podría ser objeto de propiedad intelectual debido a la novedad de la invención. A continuación se resume el avance en el desarrollo de metodologías reproducibles y eficientes para la conservación

de germoplasma, micropropagación, y transformación genética del lulo y tomate de árbol en el período 2000 y 2001. Estos avances han contando con la gentil disposición del germoplasma proveniente del Centro Frutícola Andino (CEFA) y Corpoica LA Selva, sin el cual no sería posible realizar esta investigación. El presente trabajo incluye la participación de una estudiante M.Sc., Vanessa Segovia.

Objetivo general: Ofrecer incentivos al agricultor Colombiano para el cultivo de especies oriundas de la región con un valor agregado en el mercado Nacional y de exportación, que además contribuyan con la preservación del ecosistema de laderas y la biodiversidad nativa.

Objetivos específicos: 1) Desarrollar un método eficiente de conservación in vitro a largo plazo del germoplasma de lulo y tomate de árbol; 2) Desarrollar un método eficiente de multiplicación masiva y rápida de material elite de interés comercial, mediante el uso de cultivo de tejidos *in vitro*; y 3) Desarrollar un método eficiente para la transformación genética de estas dos especies que pueda ser utilizado para la incorporación de genes de interés.

Material Vegetal:

- Selecciones de materiales comerciales de alta calidad realizadas y mantenidas *in vitro* por el Centro Frutícola Andino-CEFA. Genotipos de lulo con espinas y sin espinas.
- Genotipos facilitados por COROPOICA (Sede La Selva) de lulo con espinas y lulo "La Selva", variedad generada en ese centro resistente a nemátodos.

Resultados Año 2000.

- Se desarrollo una metodología para conservación y propagación *in vitro* de la colección de germoplasma con resultados superiores a los aplicados por CEFA y Corpoica La Selva. Esta metodología incluye la utilización de un medio diferente, y modificaciones del protocolo de propagación in vitro. Con este método se observó una mayor proliferación de raíces, desarrollo y vigor de las plantas. Esta metodología se desarrolló tanto para lulo como para tomate de árbol.
- Para poder transformar plantas, es necesario contar con un método eficiente de regeneración de plantas a partir de cualquier tejido de la planta original. Se ensayaron dos vías de regeneración; *organogénesis* y *embriogénesis somática* siguiendo protocolo reportados en la literatura. En el caso de tomate de árbol, no se obtuvo ninguna respuesta. En el caso de lulo, después de introducir varias modificaciones a los protocolos publicados, incluyendo tipo de explante, medio, pretratamiento al tejido, edad y orientación del explante, fue posible regenerar algunas plantas mediante organogénesis. En base a la falta de respuesta en tomate de árbol y los mejores resultados en lulo, se decidió temporalmente discontinuar los trabajos con tomate de árbol, y concentrar mas los esfuerzos en lulo para optimizar el protocolo y luego retomar tomate de árbol una vez que la metodología de lulo este sido establecida con alta eficiencia.

- Se realizaron ensayos preliminares de transformación mediada por *Agrobacterium tumesfaciens*. Estos ensayos indicaron que el lulo es apto a este tipo de transformación genética. Sin embargo, debido a la baja tasa de regeneración de plantas disponible a la fecha, se decidió primero optimizar el proceso de regeneración in vitro antes de proseguir con esta actividad, la cual será retomada hacia finales del 2001.

Resultados año 2001

- Después de al menos 10 subcultivos in vitro de las plantas de lulo en el óptimo establecido en el año 2000, se comenzó a observar un efecto de decoloración en las hojas, indicando un "*estrés*" en el tejido. La manifestación de los cambios en las plantas indicaron que se trataba de un efecto fisiológico del medio de cultivo in vitro, por lo que se introdujeron cambios en la composición del medio. El medio nuevo además de eliminar el deterioro fisiológico antes mencionado, acelera la emisión de raíz (haciendo más eficiente el proceso) y aumenta considerablemente el vigor de las plantas. Después de varios subcultivos en este medio, se ha podido recuperar la mayoría del material que presentaba deterioro fisiológico en el medio anterior, y podemos recomendarlo como un medio mas eficiente para fines de conservación de germoplasma y micropropagación. Recientemente Corpoica La Selva nos ha enviado otros materiales de su banco de germoplasma *in vitro*, los cuales han presentado problemas, para evaluar si con la metodología y medio optimizados en CIAT tienen una mejor respuesta. Una vez evaluados estos materiales se compartiría la información con Corpoica La Selva.
- Se realizaron un gran número de ensayos siguiendo un diseño experimental estadístico y número de repeticiones para garantizar la reproducibilidad de los resultados a gran escala. Se realizaron modificaciones adicionales al protocolo original que en el año 2000 había dado regeneración de las primeras plantas y se optimizó. En este momento se cuenta con un protocolo que puede ser utilizado para fines de transformación genética.
- Paralelamente se estableció una metodología para la transferencia del material regenerado *in vitro* al invernadero y posteriormente al campo.
- Las primeras plantas regeneradas han mostrado un desarrollo vegetativo y floración normal en el invernadero y en el campo. Las fructificación esta siendo evaluada en campo a condiciones optimas para cultivo de lulo (1700 m.s.n.m y 22°C) en la finca "LA CASONA", ubicada en el corregimiento de DAPA a 20 minutos de Cali.

Planes futuros

- Establecer un cultivo cíclico entre invernadero e *in vitro* para renovar continuamente la colección.
- Implemento y optimización del protocolo de transformación.
- Evaluación en campo de materiales regenerados en el laboratorio, y comparados con micropropagación *in vitro* y semilla sexual.

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106195

Molecular and Agro-morphological Characterization of the Genetic Variability of Soursop (*Annona muricata* L.) Accesions and related Annonaceus Species

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[1]Project SB02 -CIAT; [2]Corporación BIOTEC; [3]CORPOICA; Project funded by Colciencias

Background:

The *Annonaceae* family (common name: anonas) presents 240 species of 30 genera in Colombia, and comprises several groups important as food products, commonly known as anones (*A. squamosa*), soursops (*A. muricata*), chirimoyas (*A. cherimola*), atemoyas (*A. squamosa* x *A. cherimola*) and "anones amozonicos" (*Rollinia mucosa*), among others (Murillo-A, 2001).

Soursop (*Annona muricata* L.), because of its exquisit taste, flavor and nutritive value is the most promisory anona species in Colombia (CIAT and C. BIOTEC, 2002). Like all the *Annonas*, soursop is native to the American tropics, possibly Colombia or Brazil (León, 1968). This is why Colombia may have the world's largest genetic diversity of soursop. However the extent of this diversity is not known , and has not been used in breeding programs to improve agronomic traits either. As a matter of fact, theres is only one germplasm bank in Colombia, C.I. Corpoica Palmira Germplasm Bank (43 anonas accesions), which has not been characterized at all.

This project is the first attempt to study the genetic diversity of annonaceus species from Colombia. The objective is to know the genetic variability of the germplasm bank by applying DNA molecular marker technology, like AFLP. We would like to know if this variability is representative of Colombian diversity. Corporación Biotec and CIAT (SB02 project) are in charge of the molecular characterization. Corpoica carries out the agro-morphological characterization and a support in population genetics. The results of this project will contribute to the development of more productive, genetically diversified planting material.

Methodology:

For the genetic variability analysis, we evaluated 81 accesions from Corpoica germplasm bank and our own working collection. The 81 accessions were made of 45 entries from *A. muricata* and 36 of related *Annonaceae* species (Tables 1 and 2). We applied the method of Dellaporta et al. (1983) for DNA extraction, using PVP 1 g l^{-1} in the extraction buffer and a chloroform / isoamylalcohol (24/1) cleaning step before DNA precipitation. The AFLP protocol followed was that described by Vos et al. (1995), using AFLP Analysis System I Kit (Gibco BRL). In preliminary trials we evaluated 15 different combinations of selective nucleotides for AFLP amplification. Then, we selected three combinations that displayed the highest and "most readable" polymorphisms between soursop species and/or between Annonaceae species. They were **E-ACT, M-CAA (combination F); E-AGC, M-CTC (combination M); E-AGC, M-CAA (combination N).** Selective amplifications were size-fractionated on 6% or 4% polyacrylamide denaturing gels and visualized through silver staining. AFLP fingerprinting of each accesion were converted into a similarity matrix, based on Nei and Li index (1979). The similarity matrix was analyzed using NTSYS (Rohlf, 1994) computer program. Dendrograms were constructed by UPGMA method (Sneath and Sokal, 1973).

Results and Discussion:

In the soursop germplasm, the 45 accesions showed 141 bands for combination **F**. The number of bands per individual ranged between 41 and 58, where only eight bands were monomorphic (Fig. 1), giving a 90 % polymorphism. Fourty-one accesions presented genetic similarities higher than 0.80 (Fig. 2). Four accesions, 1919, 1959, 1958 and 1920, which are currently identified as *A. muricata* species (C.I. Corpoica Germplasm Bank), showed specially different fingerprints. Genetic similarity between 1919, 1959 and the other 41 *muricata* accesions was 0.45. Similarly, genetic similarity between 1958, 1920 and the other 41 accesions was 0.30. As we can see in Fig. 3, 1959 looks more similar to *A. montana* than to *A. muricata*, while 1958 appears more similar to putative *A. glabra* accesions, and 1920 showed more similarity to putative *Rollinia mucosa* accesions (Table 2).

Table 1. Soursop and other anona-related species accessions of the C.I. Corpoica Palmira Germplasm Bank (August – 2002).

SOURSOP ACCESIONS				
	ACCESSION	ORIGIN	PROPAGATED BY	INTRODUCTION
1.	1994	El Bolo Valle	Seed	I – 89
2.	2045	Palmira Valle	Seed	X – 89
3.	H 2	Sonso Valle	Seed	IX – 89
4.	2512	Palmira Valle UNAL	Grafting	IX – 95
5.	2511	V. Gorgona Valle	Grafting	IV – 95
6.	H 1	Sonso Valle	Seed	IX – 89
7.	2020	Buga Valle	Seed	VII – 89
8.	2016	Buga Valle	Seed	IV – 89
9.	2017	Buga Valle	Seed	IV – 89
10.	1957	Caicedonia Valle	Seed	VIII – 87
11.	1995	V. Gorgona Valle	Grafting	I – 89
12.	1983	Palmira Valle	Seed	V – 87
13.	1985	Bajo Calima Valle	Seed	V – 88
14.	1946	Alcala Valle	Seed	VII – 87
15.	1943	Palmira Valle	Seed	VII – 87
16.	2014	Barinas Venezuela	Seed	I – 89
17.	2015	Fusagasuga Cundinamarca	Seed	II – 89
18.	2036	Sonso Valle	Grafting	IX – 89
19.	2042	Sonso Valle	Grafting	IX – 89
20.	1921	Ginebra Valle	Seed	V – 87
21.	1919	Samana Caldas	Seed	V – 87
22.	2040	Sonso Valle	Grafting	IX – 89
23.	2041	Sonso Valle	Grafting	IX – 89
24.	2039	Sonso Valle	Grafting	IX – 89
25.	6	Palmira Valle	Grafting	IX – 95
26.	1918	Manizales Caldas	Seed	IV – 87
27.	4	Palmira Valle	Grafting	IX – 95
28.	2037	Sonso Valle	Grafting	IX – 89
29.	2033	Sonso Valle	Grafting	IX – 89
30.	1	Palmira Valle	Grafting	IX – 95
31.	2513	C.I. Palmira Valle	Grafting	II – 86
32.	2514	Sonso Valle	Grafting	IX – 89
ANONAS SPECIES ACCESIONS				
33.	1959 Patrón	Sabaletas Chocó	Seed	VIII – 87
34.	1958 Patrón	Sabaletas Chocó	Seed	VIII – 87
35.	1920 Patrón	Victoria Caldas	Seed	V – 87
36.	Cabeza de Negro	Desconocido	Seed	VIII – 79
37.	Chirimoya	Palmira Valle	Seed	VII – 63
38.	Anona Colorada	Desconocido	Seed	X – 36
39.	Biriba	Brasil	Seed	V – 59
40.	Anona Blanca	Palmira Valle	Seed	V – 70
41.	Anona Glabra	Baudó Chocó	Seed	Pendiente
42.	Anona Montana	Baudó Choco	Seed	Pendiente

Note: Observations made on tree and fruit morphology (Dr Robert Tulio González M, U. del Pacifico, Valle), accessions 1959 and 1920 were moved from *A. muricata* to other anona species.

Tabla 2. Soursop and other related anonas species accesions from farms and national research centres, used in the molecular characterization and available at CIAT.

ACCESSION CODE	COMMON NAME	PLACE OF ORIGIN (place and department)	PROPAGATED BY	SPECIE
AMUR V1	Guanábano, Clon Elita	Vivero Profrutales, Candelaria (Valle)	Grafting	*A. muricata*
AMUR H2	Guanábano, Clon Rosa	Yaguará (Huila)	Grafting	*A. muricata*
AMUR H3	Guanábano, Clon Cristina	Yaguará (Huila)	Grafting	*A. muricata*
AMUR H4	Guanábana, Clon Francia	Yaguará (Huila)	Grafting	*A. muricata*
AMUR M5	Guanábano	Ciénaga (Magdalena)	Seed	*A. muricata*
AMUR A7	Guanábano	Turbo (Antioquia)	Seed	*A. muricata*
ASP V20	Anona Blanca Lisa	Finca la Esneda, Guacarí (Valle)	Seed	*A. squamosa?*
ASP GN31	Anón Amazónico	Margen Río Inírida (Guainía)	Seed	*Rollinia mucosa?*
ASP VA32	Anón Amazónico	(Vaupés)	Seed	*Rollinia mucosa?*
ASP GV34	Anón Amazónico	Finca la Primavera San José (Guaviare)	Seed	*Rollinia mucosa?*
ASP VA35	Anón Amazónico	(Mitú) Vaupés	Seed	*Rollinia mucosa?*
ASP V36	Anón Amazónico	Desconocido (Donado por Cartón Colombia)	Seed	*Rollinia mucosa?*
ASP Q38	Anón	La Tebaida (Quindío)	Seed	*?*
ASP V41	Atemoya	Finca la Esneda, Guacarí (Valle)	Seed	*A. cherimolia x A. squamosa*
ASP V42	Atemoya	Finca Venecia Caicedonia (Valle)	Seed	*Rollinia mucosa?*
ASP V43	Atemoya	Desconocido, Almacenes Exito Cali (Valle)	Seed	*A. cherimolia x A. squamosa*
AMON V51	Guanábana Cimarrona	Finca la Esneda, Guacarí (Valle)	Seed	*A. montana*
AMON Vi52	Guanábana Cimarrona	Cumaribo (Vichada)	Seed	*A. montana*
AMON H53	Guanábana Cimarrona	(Huila)	Seed	*A. montana*
AMON V54	Guanábana Cimarrona	Palmira (Valle)	Seed	*A. montana*
ASP V59	Anón	Almacenes Éxito, Cali (Valle)	Seed	*A. squamosa?*
ASP CV61		Costa Pacifica (Entre Valle y Chocó)	Seed	*A. glabra?*
ASP A62	Guanabanilla	Turbo (Antioquia)	Seed	*A. glabra?*
ASP CU63	Anón Liso	Mercado de Girardot (Cundinamarca)	Seed	*?*
ASP T64	Anón	Espinal (Tolima)	Seed	*A. squamosa?*
ASP BR60		Brasil	Seed	*?*
ASP VR65		Robles (Valle)	Seed	*?*
ASP VR66		Robles (Valle)	Seed	*?*
ASP V21	Chirimoya Imbanaco	Cali (Valle)	Seed	*A. cherimolia*
ASP Q22	Chirimoya	Armenia (Quindío)	Seed	*A. cherimolia*
ASP V45	Atemoya	Finca Varahonda, Pradera (Valle)	Seed	*A. cherimolia x A. squamosa*
ASP V46			Seed	*?*
ASP V47	Chirimoya	Bolo (Valle)	Seed	*?*
ASP V48		USA	Seed	*?*

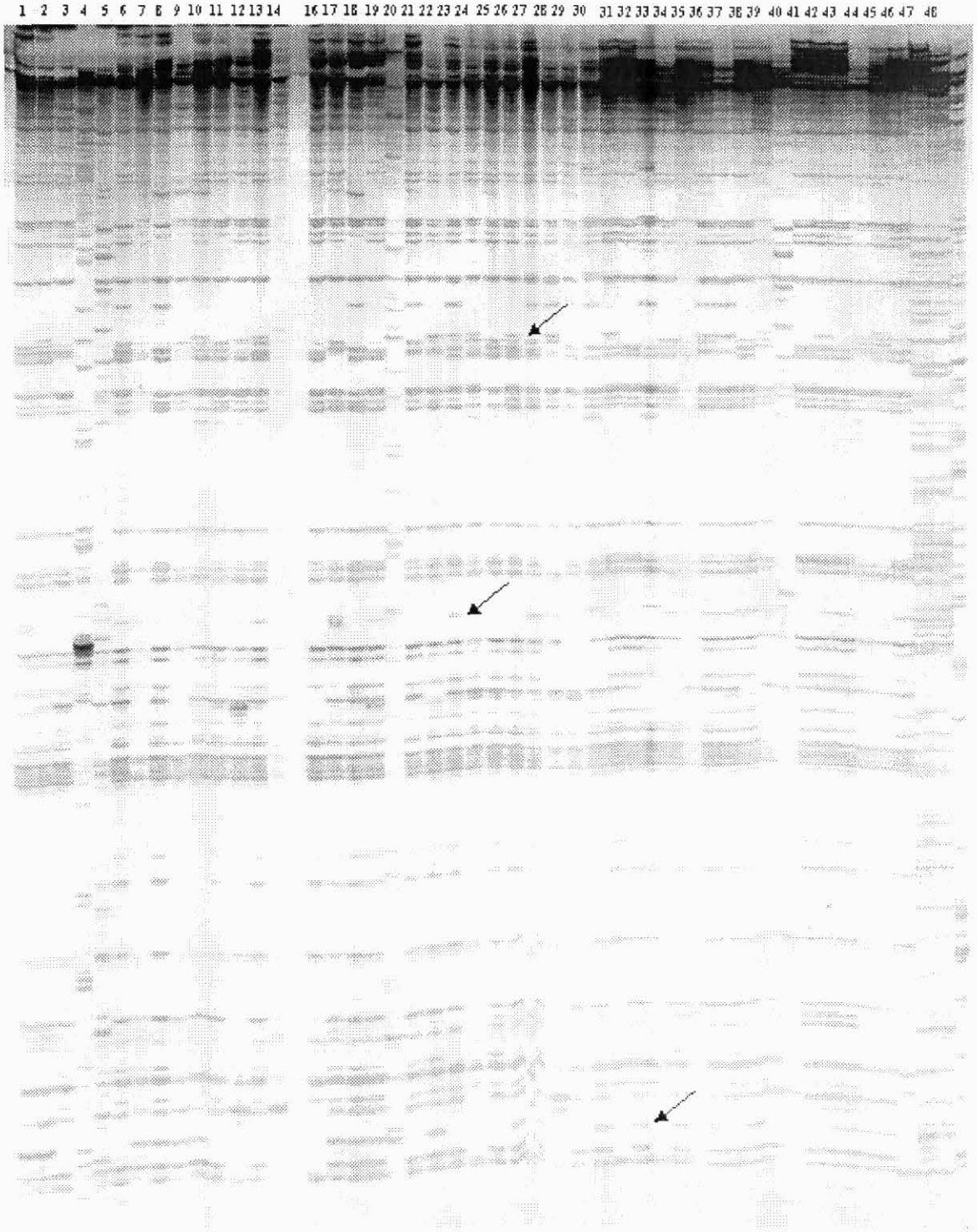

Fig. 1. Silver stained gel showing AFLP fingerprinting of *A. muricata* accesions with combination F: 1) 1985; 2) 2513; 3) 1957; 4) 1958; 5) 1959; 6) 1918; 7) C6; 8) 2014; 9) 2015; 10) 2016; 11) 2017; 12) 2040; 13) 2041; 16) AMUR V9; 17) AMUR A7; 18) AMUR M5; 19) 2033; 20) 1920; 21) 2020; 22) 2512; 23) 1994; 24) 2036; 25) 1946; 26) C1; 27) C4; 28) 2514; 29) 2037; 30) AMUR H3; 31) AMUR H21; 32) AMUR V1; 33) AMUR H35; 34) 1943; 35) 2039; 36) H2; 37) H1; 38) 1983; 39) 2045; 40) 1919; 41) AMUR V1; 42) AMUR H2; 43) AMUR H4; 44) 1921; 45) 1995; 46) AMUR H2; 48) ASP GV34. Arrows indicate polymorphic bands.

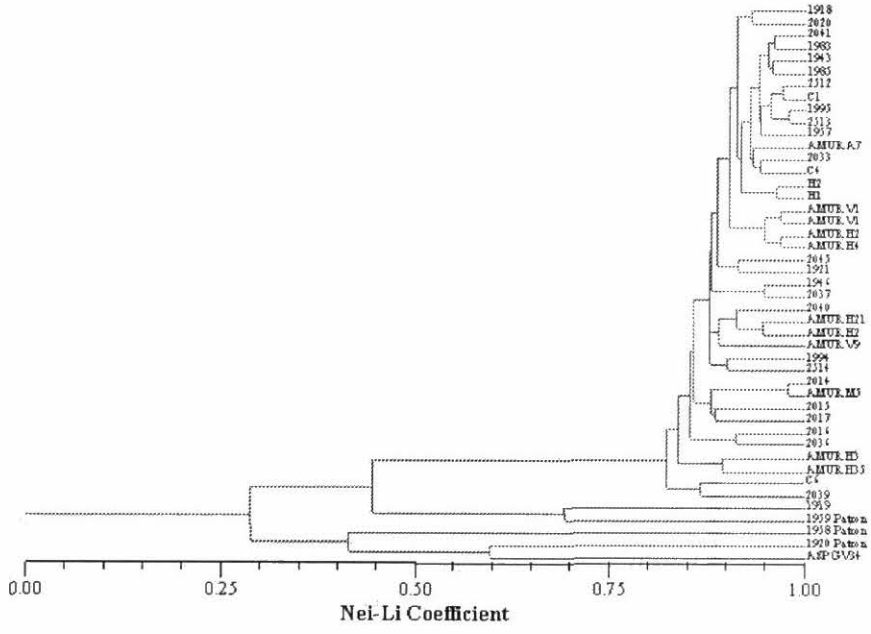

Fig. 2. Genetic similarity between *A. muricata* accesions, based on AFLP fingerprinting with combination F.

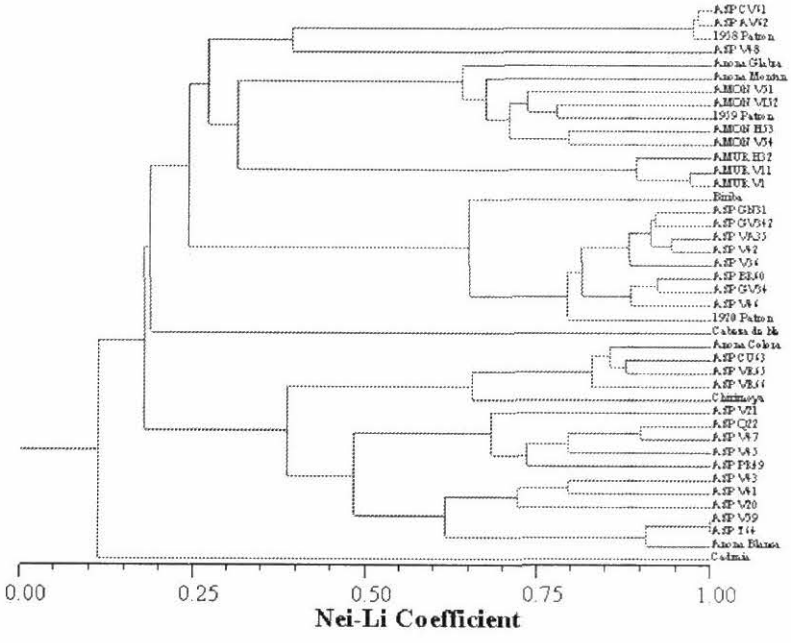

Fig. 3. Genetic similarity between anona accesions, based on AFLP fingerprinting with combination M.

Soursop accesions analysed with combination **F** presented low genetic variability, compared to the variability observed by *A. montana* and putative *R. mucosa* accesions (Fig. 2 and 3). The development of the crop in Colombia, and the exchange of germplasm by soursop growers, has made possible the trade of seeds adapted to different agroecological zones. This may be one reason why, in our analysis, 2014 and AMUR M5 from Barinas (Venezuela) and Ciénaga-Magdalena (Colombia), respectively, presented a similarity higher than 0.85 with 2015 and 2017, accesions collected from Fusagasugá-C/marca and Buga-Valle, respectively (Fig. 2).

We observed genetic variability between accessions that originated from seeds of the same tree (siblings) for instance there was variability between AMUR H2-1 and AMUR H2 as well as between AMUR H3 and AMUR H3-5 (Fig. 2). This observed variability could be attributed to natural out-crossing on account of the protogyny in the soursop flowers (Escobar and Sánchez, 1992). To arrive at a definitive confirmation of the status of variability between siblings in soursop however, it is suggested that an in-depth molecular marker analysis using AFLPs for instance, should be carried out.

In general, in the *Annonaceae* germplasm evaluated there was high genetic variability (Fig. 3). Four groups were related at 0.20-0.25 similarity: *A. glabra*-like accessions; *A. montana* and *A. muricata* accessions; accessions similar to "anones amazónicos"; chirimoyas, atemoyas and anones-like accessions.

Parallel to molecular characterization analysis, we are evaluating *Annonaceae* species as potential rootstocks for *in vitro* propagation of selected soursop clones (see "Optimization of the *in vitro* propagation methodology of selected clones of soursop (*Annona muricata* L.) and evaluation of the compatibility between different combinations of scions and rootsocks micrografted *in vitro*", this Annual Report). Previous observations indicate that not all accessions are usable as rootstocks. For instance, soursop scions respond differently to different rootstocks of *Annona montana*. Therefore it might be convenient, due to the intrinsic genetic variability (Fig. 3), to select, based on fingerprints, those accessions best suited for rootstocks.

Finally, one accession of *Annona glabra* from C.I. Corpoica Germplasm Bank appeared more related to *A. montana* accessions (genetic similarity of 0.65) than to putative *A. glabra* (similarity around 0.30). It's therefore convenient to review, based on fingerprints, the present classification of several accessions in the bank.

Conclusions:

- Each soursop accession showed a different AFLP fingerprinting with combination F. This molecular marker could be used for the identification of soursop clones and future varieties.
- Soursop accessions conserved at C.I. Corpoica Palmira germplasm bank presented low genetic variability with combination F. It may be convenient to collect new germplasm.
- It is reccomended to check the identification of at least four accessions: 1920, 1959, 1958 and *A. glabra*.

Future plans:

- Complete the genetic variability analysis of anonas accessions with two different combinations of primers (combinations M and N).
- Evaluate the genetic variability of siblings by AFLP markers.

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Optimization of the *in vitro* propagation methodology of selected clones of soursop (*Annona muricata* L.) and evaluation of the compatibility of different scion and rootstock combinations for *in vitro* micrografting

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[1]Project SB02; [2]Corporación BIOTEC; [4]Project supported by Pronatta; [5]Project supported by Fundación Banco de la República

Background:

Between 1996 and 2001, we developed a methodology for the *in vitro* clonal propagation of elite selections of soursop (or guanábano in Spanish, *Annona muricata* L; Royero *et al.*, 1998), a fruit tree that is native to tropical America. The developed methodology allows a rapid clonal multiplication of selected trees and the production of disease-free plants. The plants produced through *in vitro* micrografting have been found to develop normal and healthy growing trees that produce the first fruits 15 months after planting (MAP) in the field.

The developed micrografting technology fulfills all the requirements for ridding planting materials of diseases. However before it can be applied for large-scale propagation, some critical steps in the propagation process have to be optimized.

In 2002 several steps involved in the propagation were reevaluated and media modifications were tested. Furthermore, the compatibility of scions from different selected clones of soursop micrografted over different rootstocks from the same and/or other related annonaceus species were studied at the laboratory and greenhouse levels.

Methodology:

The general methodology for *in vitro* propagation of soursop through micrografting has been described in previous reports (Royero *et al.* 1998 and 1999).The composition of the media and solutions used in the whole process is presented in the tables 1 and 2.

Table 1. Composition of the culture media previously used and of the new improved media (in bold) for the propagation process of soursop through micrografting

Use	Medium denomination	Salts	Sucrose	B5 Vitamins	Casein-Hydrol.	BAP	GA_3	PVP	Agar[1]
		g/L	g/L	g/L	g/L	mg/L	mg/L	g/L	g/L
Culture of shoot pieces for axillary bud induction	M-I	WPM[2]	20			1			4.8
	RO-BAP 1	MS[3] 1/2	20	0.112	0.2	1			4.2
Culture of shoot pieces for axillary bud elongation	M-II	WPM	20			0.2			4.8
	RO-BAP 0.2	MS 1/2	20	0.112	0.2	0.2			4.2
Seed germination	M-III	B5[4]	20				0.5		4.8
	RO 1/2 GA3	MS 1/4	10	0.056	0.2		1		4.2
Micrograft culture and development	M-IV	WPM	20	0.112					4.8
	RO 1/2	MS 1/4	10	0.056					4.2
Micrografting solution	M-V	WPM	20					1	
	T3	WPM	20		0.2	0.2		1	

1 From Duchefa, Netherlands
2 Lloyd y McCown 1981, purchased from Duchefa, Netherlands
3 Murashige and Skoog 1962, purchased from Duchefa, Netherlands
4 Gamborg et al. 1968, purchased from Duchefa, Netherlands

Table 2 . Composition of the different antioxidant treatments evaluated during micrografting of combinations of scions of the clone Rosa with rootstocks of Rosa, *A. montana* and *A. glabra*.

Treatment	Basal medium	Antioxidant (g/l)	Additional compounds	Growth regulator mg/l
T0	Half concentrated MS[2] Salts	PVP[3] 1	-	-
T1	WPM[1] Salts	PVP 1	Sucrose 2%	-
T2	WPM Salts	PVP 1	Casein hydrolysate 200 mg/l Sucrose 2%	-
T3	WPM Salts	PVP 1	Casein hydrolysate 200 mg/l Sucrose 2%	BAP 0.2

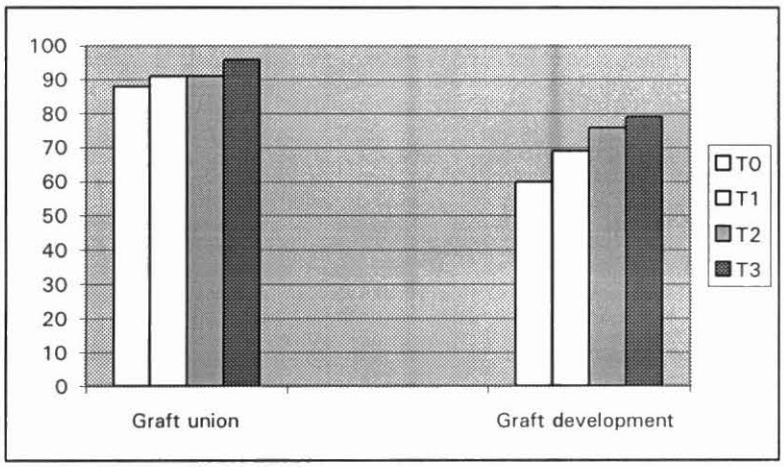
1 Lloyd and McCown, 1981
2 Murashige and Skoog, 1962
PVP = Polyvinylpyrrolidone

Results:

Evaluation of different antioxidant solutions used during the process of *in vitro* micrografting

Cut explants of woody species cultivated *in vitro*, are characterized by the exudation of phenolic compounds that oxidize in contact with the air, forming a dark precipitation. This phenomenon is known as phenolization or oxidation and when it is severe, can cause the explants to die. Soursop as a woody tree is not an exception, and tissue phenolization greatly affect the success of micrografting. We have in the past years tested the effect of different antioxidants in reducing phenolization during the micrografting process, and found polyvinylpyrrolidone (PVP) as being the best. In 2002 we investigated the effect of different modifications to the micrografting solution used on the efficiency of graft union, and in supporting growth of the micrografted scion. The results are shown in fig. 1. Compared to the antioxidant solution used before, rates of graft union and development of the scion after micrografting were improved by using micrografting solutions that in addition to the antioxidant were supplemented with WPM salts, sucrose and casein hydrolysate. But the best results regarding the scion development were obtained when the antioxidant solutions were supplemented additionally with the growth regulator BAP. This growth regulator or the combination of it with the other components of the solution also promoted a fast growth of the scion allowing the plantlets to be transferred to the greenhouse in less than 6 weeks after micrografting.

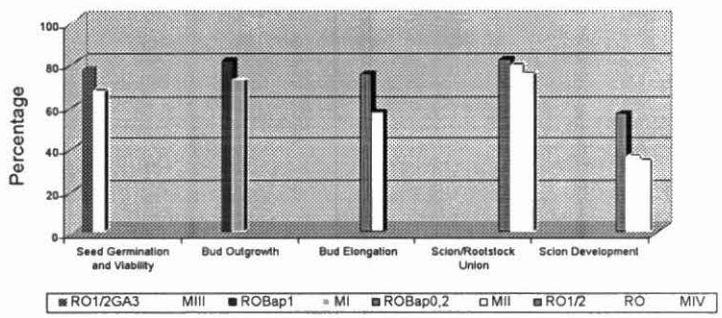
Figure 1. Evaluation of the effect of different antioxidant-solutions used during micrografting, on the graft union and on the development of the micrografted bud. Buds of the clone Rosa were micrografted on rootstocks of Rosa, A. montana and A. glabra. The composition of the antioxidant solution is explained on table 2.



Evaluation of the culture media used

The *in vitro* phase of the propagation methodology of soursop through micrografting consists of many step: (1) seed germination *in vitro* (for rootstock production); (2) induction of growth of axillary buds, (3) elongation of axillary buds (for scion production), (4) culture of micrografts and (5) culture of mother micrografts (*in vitro* micrografts used as source of buds for the production of new micrografts). Each of these steps required a different culture medium of a different composition (Table 1). In 2002 these culture media were revised and their preparation simplified by using only one source of a ready to use salt mixture as the basal medium. The newly developed media can be prepared easier and faster. Also by using them an improvement on the success rate of every step involved in the propagation process was achieved (Fig. 2) , allowing the performance of the propagation with more efficiency than before. The most important modification of the culture media is the use of the MS-salt mixture in half or one-quarter concentration, instead of the complete WPM salts.

Fig.2 Comparison of the overall success rate achieved in the different steps of the propagation of soursop through *in vitro* micrografting with the old and the new culture media.

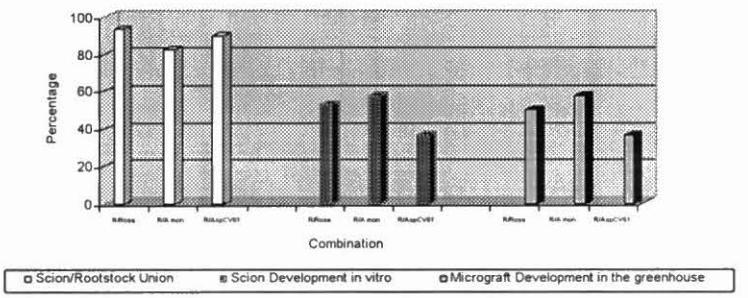


Use of rootstocks of other *Annona* species for the production of more vigorous, disease resistant and widely adapted soursop trees

The use of rootstocks of different genotypes or species from that of the scion for the production of vigorous, widely adapted or disease resistant plants is a common practice in fruit tree propagation. In soursop this avenue has been largely under-exploited.

We are investigating both at the laboratory and greenhouse levels, the compatibility of scions of different selected clones of soursop and different rootstocks of the same species and of the related species *A. montana* and *A. glabra*. The results of the micrografting experiments of rootstocks of these species with scions of the clone Rosa are presented in the figure 3. Regarding micrograft union (measured 15 days after micrografting) no significant differences were found between the different rootstocks. However regarding the development *in vitro* and in the greenhouse of the micrografted buds, significant differences were found. Surprisingly, it was the combination Rosa/A. montana, and not the micrografts of the scion of Rosa over its own rootstocks that was the combination that has shown the highest frequency of development. This combination is also the one that shows the highest growth rate in the greenhouse (data not shown).

Fig.3 Percentage of union and development *in vitro* and in the greenhouse of scions from the clone Rosa of soursop micrografted over rootstocks of Rosa, *A. montana*, and *A. glabra*.



Total number of plants produced from different combinations of scion and rootstocks

In 2002, a total of 1871 micrografted plants were produced from different combinations of scion and rootstocks (Table 3). All of these will be planted in the field for further evaluation of their agronomic performance under different agroecological conditions.

Conclusions:

With the newly evaluated media and solutions, an improvement on the success rate of every step of the propagation process was achieved

The survival of the micrografted plants in the greenhouse depends largely on the development achieved by them in the *in vitro* phase. With the combination Rosa/A. montana V54 the highest survival rate so far in the greenhouse, 68.4%, has been achieved.

The *in vitro* micrografting propagation methodology could be applied to 3 different soursop clones (all the clones evaluated) and 5 different rootstocks from the same and other annonaceus species (also all the rootstocks evaluated).

Efficiency of development of micrografted plantlets *in vitro* should be improved in order to apply the technology for massive propagation of soursop clones.

Table 3. Overall number of micrografts made, and micrograft development efficiency with different combinations of soursop scions and rootstocks of soursop or related annonaceus species between June

2001 and May 2002. Cristina, Elita, Rosa and Francia are selected clones of soursop (*Annona muricata* L.).

Scion/Rootstock Combination	Total of micrografts made	Micrografts showing graft union	Micrografts showing bud development	% of micrograft development
Rosa/*A. montana* V54	38	29	26	68.4
Elita/Rosa	260	207	157	60.4
Rosa/Rosa	289	268	174	60.2
Rosa/Elita	911	835	542	59.5
Cristina/Rosa	310	245	182	58.7
Francia/Cristina	178	150	99	55.6
Rosa/*A. glabra* CV61	282	258	154	54.6
Rosa/Cristina	100	90	54	54.0
Elita/Cristina	512	434	264	51.6
Cristina/Elita	273	234	136	49.8
Rosa/*A. montana* V51	124	93	55	44.3
Cristina/Cristina	100	39	28	28.0
TOTAL	**3377**	**2882**	**1871**	**55.4**

Future plans:

- To improve the methodology of production of rootstocks *in vitro*, which is the most limiting step in the process of scaling up of the propagation methodology.
- To include more elite selected clones and more rootstocks in the propagation and evaluation process.

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Optimisation of the hardening process of *in vitro* micrografted plantlets of soursop (*Annona muricata* L.) under greenhouse conditions using different substrates

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[1]Project SB02 CIAT; [2]Corporación Biotec; [3]Project supported by Pronatta; [4]National University of Colombia, Palmira; [5]Independent Consultant.

Background:

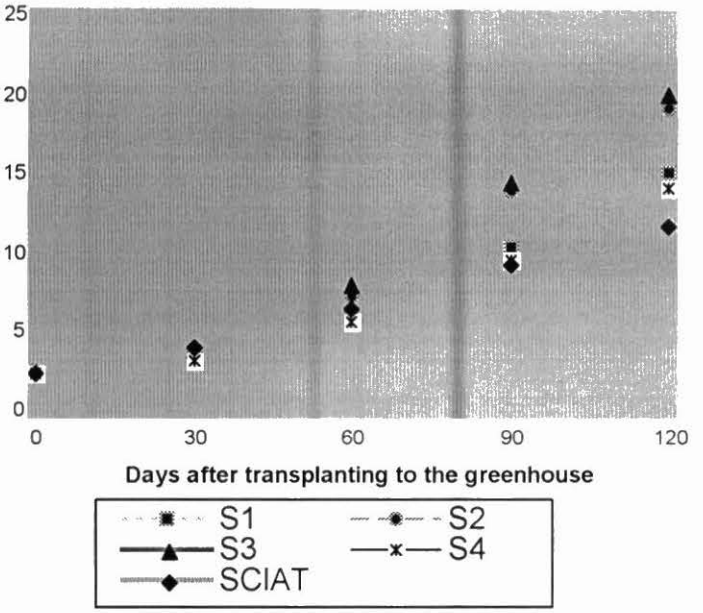
The hardening process of *in vitro* propagated plants in *ex vitro* conditions, normally carried out in a greenhouse, is one delicate step in any propagation process and is usually characterized by high mortality rates of the plantlets. In past years we have made important advances in the development of methodologies for *in vitro* clonal propagation of soursop (*Annona muricata* L.), but the hardening of micropropagated plantlets in the greenhouse has not received enough attention. During the year 2002 we continued with the optimisation of this process by the use of different combinations of substrates that are by-products of the sugar and paper industry in the Valle del Cauca region of Colombia. Some of the substrate components investigated were reported by Bruzon (1997) as having excellent properties for sustaining plant growth under greenhouse conditions. Additionally, the effect of microrrhization of the plants on their survival and vegetative growth is also being investigated.

Methodology:

Two-month old micrografted plantlets of the clone Rosa over the rootstock Elita were used. They were transferred to the greenhouse and planted in different substrates that contained CIAT soil, sand, cachaza (filter cake), coal ash, rice shells or pine bark chips. Most of these components are by-products of the sugar or paper industry. Alternatively, the plants were inoculated with vesiculo-arbuscular (VA) micorrhizal fungi (Table 1) in D16 Deepot™ containers (Hummert International).

Table 1. Description of the vesiculo arbuscular micorrhizal fungi used (CIAT 2000)

No	Mycorrhiza	Altitude (m)	Host Plant	Collector	P (mg/kg)	pH	Al (meq/100g)	MO (%)	Cant (e/g)*
1	*Gomus. desertícola*	200	Grasses	Sieverding	1	4.5	2.2	3.3	610
2	*Gigaspora margarita*	200	Grasses	Spain		4.5			42
3	*Gigaspora rosea*		Glycine max	D. Pellet	48	7.3	0	1.9	40



Results

Survival of *in vitro* micrografts planted on different substrate combinations in the greenhouse.

A very highly significant effect of the substrate type on the survival and growth of the micrografted plants in the greenhouse was found. While only less than 50% of the plants survived when planted on CIAT soil, substrates containing "cachaza" and coal ash, showed survival rates of over 70%. The highest survival rate was achieved with the substrate combination S2 (Table 1) which contained 3 parts "cachaza", one part of coal ash and one part *Pinus* bark chips. The highest growth however was shown by the micrografts planted on substrate S3 (Figure 1)

The high survival rate achieved on the S2 substrate can be attributed not only to the nutritional properties of the substrates (its high contents of phosphorus, nitrogen, calcium and organic matter; Table 2), but also to its better physical properties as compared to CIAT soil. These results confirm observations made by Bruzón (1997) with similar substrates.

Table 1. Survival rate of micrografts planted on different substrates.

Substrate	Component Proportion				Survival rate (%)
	CIAT Soil	"Cachaza" Filter cake	"Carbonilla" Coal Ash	Pinus Bark chips	
S1		3	1		71.43
S2		3	1	1	95
S3	1	2	1	1	76.67
S4	1	3	1		80
CIAT-Soil	soil-sand 1:1				48.89

Height, number of leaves, dry matter, leaf area and root volume of micrografts after 4 moths of culture on different substrates in the greenhouse:

Significant differences between substrates for plant height (Pr = <0.0001), but not on number of leaves (Pr = 0.5196 and 0.2398; Figure X) were found. Through Duncan Test we could identify S2 and S3 as the best combinations (Table 3). In contrast, through LSD test, a positive effect of substrates on dry matter, foliar area and root volume was found, with the substrates S2, S3 and S4 being the best in this regard.

Figure. 1 Height reached by the scion after 0, 30, 60, 90 and 120 days of culture of the micrografts on different substrates in the greenhouse. SCIAT = CIAT soil.

Table 3. Mineral content, nutritional and physico-chemical properties of the components and the substrates used.

Substrate	N	P	K	Ca	Mg	MO	C.O	C/N	PH	Al
	%			g/kg		(%)				(meq)
Rice shells	0.45	0.14	0.337	0.81	0.61	67.90	33.95	75.87	-	-
Pinus Bark	0.26	0.02	0.005	2.97	0.44	76.00	38	145.04	3.96	3.17
Coal Ash	0.19	0.02	0.004	1.09	0.27	1.80	0.9	4.83	6.91	-
CIAT Soil	0.17	0.02	0.010	0.72	0.20	3.60	1.8	10.35	5.35	0.36
"Cachaza"	0.71	0.88	0.086	4.42	1.97	14.00	7	9.89	6.85	-
S1	0.51	0.69	0.063	3.81	1.54	9.80	4.9	9.63	7.04	-
S2	0.53	0.57	0.074	4.38	1.51	21.20	10.6	20.08	6.11	-
S3	0.31	0.32	0.043	2.97	0.85	12.00	6	19.23	6.51	-
S4	0.46	0.45	0.053	3.23	1.15	8.40	4.2	9.05	6.72	-

Table 4. Dry matter, leaf area, root volume, plantlet height and number of leaves of Rosa/Elita micrografts, after four months of culture in the greenhouse on different substrates.

Substrate	Volumetric Mixture (V/V)					Dry matter	Foliar area	Root volume	Height	Leaf number
	Soil	Sand	Cachaza	Coal Ash	*Pinus* bark chips	(%)	(cm^2)	(cm^3)	(cm)	
S1			3	1		19.39ab	168b	1.5b	6.76b	4.58a
S2			3	1	1	**20.30a**	**306.7a**	**3.5a**	**8.44a**	**4.68a**
S3	1		2	1		20.03ab	266.97a	3.12a	7.96a	4.64a
S4	1		3	1		18.35ab	318.72a	3.12a	6.75b	4.64a
CIAT soil	1	1				17.57b	120.55b	2.12b	5.66b	4.33a
Average						19.13	236.18	2.67	7.11	4.57
C.V.(%)						9.06	25.33	32.77	25.63	26.71
Pr>F						0.2014	0.0016	0.0376	<0.0001	0.5196

Values for each parameter within columns followed by a similar letter are not significantly different from each other (Pr≤ 0.05, Duncan's Multiple Range Test)

Based on these results, substrate S2 has been chosen for culturing all micrografts produced in the greenhouse. Micografts cultured on this substrate after 4 months attain sizes sufficient for surviving transplanting to the field.

Effect of the innoculation of micrografted plants with Vesiculo-Arbuscular Micorrhizal Fungi in the greenhouse

Four months after transfer to the greenhouse and inoculation, a low colonization of roots (between 12 and 25% of the evaluated rootsegments) was found. This colonization did not have any significant effect on micrograft height (Pr=0.1973). However its effect on the number of leaves was significant (Pr=0.0184).

Through microscopic observations, a high proportion of collapsed spores were found. Its presence as well as the low root colonization can possibly be caused by the high content of phosphate in the substrates or its high pH (Koide and Schereiner, 1992)

Conclusions:

An improvement of survival and growth rate of soursop micrografted plants was achieved through the use of substrates containing "cachaza", coal ash and *Pinus* bark chips (substrates S2, and S3). These substrates also showed excellent physico-chemical properties.

No effects of VA-Micorrhizal fungi on micrograft growth were observed possibly due to the high phosphate contents of the substrates and their high pH.

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Field evaluation of the agronomic performance of soursop (*Annona muricata* L.) clones propagated through *in vitro* micrografting

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Background:

Between 1996 and 1999, a methodology was developed for *in vitro* propagation of selected clones of soursop (or guanábano in Spanish, *Annona muricata* L). This methodology consists in the *in vitro* micrografting of buds over rootstocks obtained from *in vitro* germitated seeds. The buds used are obtained either from shoots cultured *in vitro*, isolated from clones growing in the greenhouse or from micrografts produced previously (cyclic micrografting). *In vitro* propagation of plants offers many advantages over traditional methods of vegetative propagation, however some propagation methodologies have been shown to produce a variable proportion of abnormality in the growth of the plants, known as somaclonal variation. Somaclonal variants can be a real problem when *in vitro* propagation is used for supplying commercial plantations with planting material.

The occurrence of somaclonal variations in *in vitro* propagated plants has been attributed to genetic or epigenetic changes caused by the use of methodologies which involve cellular dedifferentiation, callus formation or direct production of adventitious buds.

Since in the soursop propagation process no adventitious formation of new buds is involved, and only the "natural" system of axilary bud re-growth is used for the production of new buds, no genetic changes are expected to occur in the propagated trees. However the induction of epigenetic changes such as pleiotropy or juvenility or simply root malformation (see George, 1993), can not be excluded.

In order to test if the developed propagation methodology is useful for producing true-to-type planting material, we started the evaluation of trees propagated through *in vitro* micrografting at CIAT and in farms located in different soursop producing zones of Colombia in February 2000.

Methodology:

The evaluated plants were obtained from the clone Elita (Rios Castaño and Reyes, 1996) micrografted over rootstocks of the same variety. These were planted in January 1999 in farms belonging to experienced soursop growers located in Huila and Valle or later at CIAT in February of 2000. At CIAT besides the micrografted plants, other plants produced through the traditional grafting method (kindly provided by the Profrutales nursery) were planted. Micrografted plants were 8 months old while normally grafted plants were 10 months old at the time of planting at CIAT. Preliminary data on parameters related to the vegetative growth of the trees, flowering, fruit set, production, and fruit quality have been collected while more detailed data collection is on going.

Results:

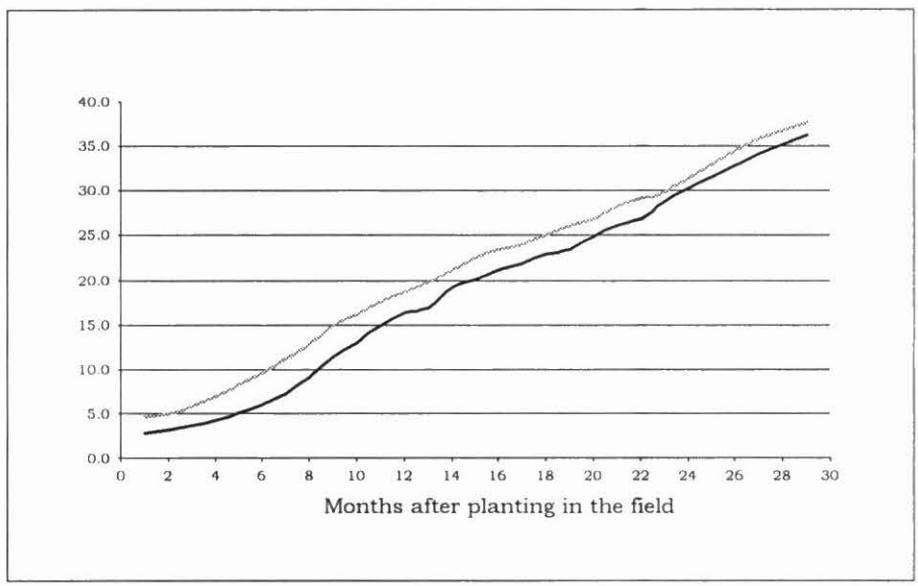
Comparison of effects of *in vitro* micrografting and traditional grafting methods on the vegetative growth of trees

In order to study if plants produced through *in vitro* micrografting are different from those produced through traditional grafting methods, the vegetative growth, fruit production and fruit quality of trees from the same genetic background and propagated through both methodologies are being evaluated at CIAT.

Tree height, volume of the canopy and perimeter at the grafting site, were taken as indices of vegetative growth during the first 12 months. But because the micrografted plants have to be pruned starting from the 12th month after planting (MAP) in order to initiate tree formation, from this time onwards only the perimeter of the stem could be taken into account, in order to have a reliable estimate of tree growth.

Until 30 MAP, the growth rates of the tress propagated through both systems are the same in the field. (Fig. 1). The small differences in size of the stems shown by the different propagation methodologies can be attributed to the differences in age and size of the trees at the time they were planted in the field.

Figure 1 Comparison of the vegetative growth of trees of the combination Elita/Elita propagated *in vitro* by micrografting (darker line) and through traditional grafting methodologies (lighter line), planted at CIAT in February 2000.



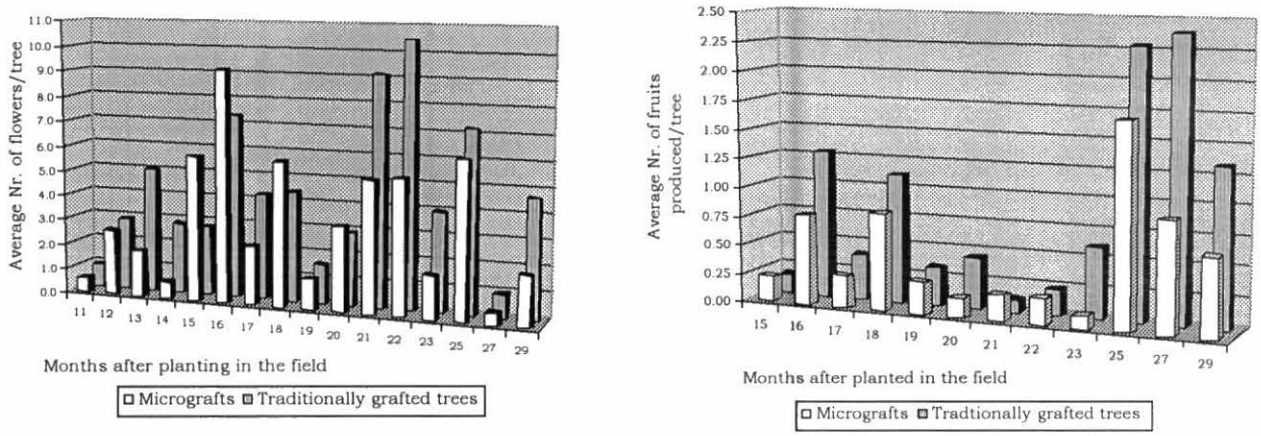
Comparison of flower set and fruit production of trees propagated by *in vitro* micrografting and by traditional grafting methods

Just as was the case with trees propagated by traditional methods, micrografted trees initiated flowering at 11 MAP and produced the first fruits at 15 MAP in the field. Trees propagated by both methods showed a cyclic behavior in flowering and fruit production, which possibly coincided with the seasonal climate changes at CIAT (Figure 2).

Although these are preliminary results because soursop trees stabilize their production only after 7 years of planting, the measurements made so far, led to the conclusion that

the plants propagated through *in vitro* micrografting suffered no delays in flowering and fruit production when compared to those propagated through the traditional methods.

Fig. 2 Flower and fruit production per tree of Elita/Elita micrografted plants planted in the field in



February 2000.

Field evaluation of new combinations of scions from selected clones and rootstocks of soursop and related species

Hitherto, only plants from one combination of scion and rootstock (Elita /Elita) have been evaluated under different field conditions. During the first half of 2002, a total of 11 new combinations were planted at Yaguará (Huila) and La Esneda (Valle; Table 1). Additionally, more than 1400 trees of similar combinations will be planted in other locations at the end the year 2002 and the beginning of 2003.

Conclusions:

No genetic or epigenetic modification, which can be attributed to the propagation process, has been observed in any of the trees propagated through the *in vitro* micrografting method.

The *in vitro* micrografting propagation procedure is a safe method for producing genetically uniform and disease-free planting materials of this species.

Table 1 Micrografted plants of different combinations of scion and rootstocks planted for field evaluation during the year 2002 in farms belonging to experienced soursop growers

Scion	Rootstock	Quantity of plants
San Francisco, Yaguará - Huila		
Elita	Cristina	84
Elita	Rosa	68
Rosa	Elita	43
Rosa	Cristina	42
Elita	Elita	19
Cristina	Rosa	13
Cristina	Elita	9
Cristina	Cristina	8
Elita	*A. montana*	8
Rosa	Rosa	4
Rosa	*A. montana*	2
Total		**300**
La Esneda - Valle		
Cristina	Rosa	20
Cristina	Elita	20
Rosa	Elita	20
Elita	Rosa	20
Total		**80**

Future plans:

- The project from which this field evaluation has been funded (PRONATTA project No. 981763225) comes to an end in December 2002. This activity will be continued if additional funds are received.

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Genetic diversity of *Ralstonia solanacearum*, causal agent of banana bacterial wilt, and its control

Introduction

Plantain is a staple food in the Colombian household basket. Exports are an important source of foreign exchange for the national economy. The crop is grown on 350,000 ha throughout Colombia. Production exceeds 2.5 million tons per year, with 99% destined for the domestic market and the rest for export. There does however appear to considerable potential for export as world demand is increasing. For the major production areas—the Coffee Zone, inter-Andean valleys, Colombian Caribbean Region, and the Eastern Plains—the crop proved to be a viable alternative at times when other crops have faced serious problems of sustainability. Of particular importance in areas with high levels of unemployment is its high labour requirement of 100 working days per year per hectare. ,

The central Coffee Zone of Colombia (Departments of Valle, Risaralda, Quindío, and Caldas) is the biggest plantain producer in the country, with an estimated area of 40,000 ha. In this region, together with other plantain-producing areas, associations of small farmers are interested in linking with the market economy and producing cash crops, including plantains.

Moko or banana bacterial wilt, caused by *Ralstonia solanacearum*, is a devastating disease of plantain. It is found throughout Colombia and cuases and estimated annual loss of about US$5.8 million. The problem is compounded by environmental deterioration caused by indiscriminate use of toxic substances such as formol as control agents.

The only controls that farmers currently use are clean seed and cultural practices that are too short term to be really effective in eradicating or detaining the disease.

Objective

This study aims to protect plantain and banana crops against *moko* on a national scale. If the disease's progress is not stopped, production will deteriorate and may reach the point where it is not economically viable.

Materials and methods

Surveying and sampling. In two samplings, we visited 36 plantain farms in the Municipalities of Montenegro, Quimbaya, La Tebaida, and Armenia (all in Quindío). Each farmer was asked a set of 40 questions. Their answers were used to describe management practices use in plantain and their effect on *moko* disease. To isolate *Ralstonia solanacearum*, the causal agent of *moko*, we took 144 samples, involving plant tissues (137), soil (146), water from reservoirs and streams (18), and insects (3).

Tissue samples. Fragments were cut from tissue samples in which symptoms of the disease were evident and from healthy tissue. The fragments were placed in beakers sealed with gauze and put into de-ionized water for 15 min. They were then disinfected by immersing in a solution of 1% NaOCl for 2 min, and washed in sterilized distilled water to remove the solution. The tissue fragments were then macerated in a sterilized solution of 1 L of distilled water; 50 mM phosphate (pH = 7.0); 4.26 g Na_2HPO_4; and 2.72 g KH_2PO_4. The macerated tissue was left for 15 min, before culturing each sample in duplicate in tetrazolium chloride (TZC) medium a differential for *R. solanacearum* and made up of 10 g peptone; 1 g hydrolyzed casein; 0.5 g glucose; and 17 g agar.

A stock solution of 2,3,5 triphenyltetrazolium chloride at 0.5 g/100 mL was prepared and sterilized separately from the TZC medium. At the time of use, 3 mL were added per 100 mL of medium.

Water samples. The water samples were diluted in sterilized distilled water by taking 1 mL of sample water and adding it to 9 mL of distilled water. Dilutions at 10^{-1} and 10^{-2} were cultured in TZC medium.

Insect samples. The insect samples were processed as for the tissue samples described above.

Soil samples. The soil samples were left to dry first by taking them out of the bags in which they were collected and exposing them to room temperature. Once dried, the samples were passed through a 2-mm sieve, and 10 g were taken from each sample and added to an Erlemeyer flask containing 100 mL of sterilized distilled water. The samples were agitated for 20 min, and dilutions then carried out by adding 1 mL of sample to 9 mL of sterilized distilled water to a dilution of 10^{-3}.

From each dilution, 0.1 mL was taken, and dilutions of 10^{-2} and 10^{-3} cultured in TZC medium.

Purification. To isolate *Ralstonia solanacearum* we first identified and purified suspect colonies that grew in the TZC medium by culturing them in fresh lots of the medium, using the drop method of culturing. The bacteria were evaluate, using biochemical tests, to see if they belonged to this genus.

Biochemical tests. Once purified in the TZC medium, the bacteria were cultured on nutritive agar to identify *R. solanacearum* bacterias and duplicate samples subjected to the following biochemical tests:

- Oxidase test, to which *R. solanacearum* is positive.
- 3% KOH test to which *R. solanacearum* is positive, indicating that is

gram negative.

Conservation. The strains obtained were conserved, either by lyophilization or kept in inclined tubes containing nutritive agar.

Results and conclusions

***Isolates of* Ralstonia solanacearum.** We identified 73 strains of *R. solanacearum* (Tables 1 and 2) from 144 samples, of which 61 (42.3%) were positive. We obtained a larger percentage of isolates from the soil samples, indicating that the bacterium is probably found in abundance in soil.

In contrast, we obtained a smaller percentage of isolates from the tissue samples, probably because either the plants were in an advanced state of infection or saprophytic bacteria that grow in sick tissues had inhibited the pathogen, hampering its isolation.

Soil samples that were positive for *R. solanacearum* were from around plants infected with *moko*, at 0-30 and 60 cm deep, 5 and 10 m up the slope, and 10 and 20 m down the slope from the sampling focus. The bacterium was also isolated from soil treated with formol and covered with plastic and from soil treated with formol 1 year ago.

The pathogen was also detected at sites that (1) had not been under plantain cultivation for 6 months, and 2 and 4 years, and (2) had been treated with Basamid®.

Table 1. Obtaining isolates of *Ralstonia solanacearum*, causal agent of banana bacterial wilt, from different tissues of infected plantain plants.

Tissue	Samples with *R. solanacearum* (no.)	Samples with no *R. solanacearum* (no.)
Stems	5	8
Seedling	1	1
Rachis	1	0

Table 2. Obtaining isolates of *Ralstonia solanacearum*, casual agent of banana bacterial wilt, from water samples.

Sample	Samples with *R. solanacearum* (no.)	Samples with no *R. solanacearum* (no.)
Stream or spring	3	2
Lake	1	0

Puddles on light-plane runway	0	1
Water supply system	3	5

Ralstonia solanacearum was also isolated from the following field plants and crops: cadillo, *Emilia* spp., Ciperaceae, lechuguilla, nightshade, arracacha, and maize.

Surveys. The farmer surveys (Table 3) indicated that:

- Over the last 5 years, they have had plants infected by *moko.*
- Tools, transport of seedlings, and stream water disseminate the disease.
- Very few control methods reduce pathogen inoculum in infected soil.

Table 3. Incidence of *moko* or banana bacterial wilt (*Ralstonia solanacearum*) and management practices in the Department of Quindío, Colombia.

Diagnosis	Value
Study on losses	
Interviewed farmers (no.)	21
Banana farmers reporting *moko* (%)	95
Area infected by *moko* (%)	11
Increases in infected area over the last 5 years (%)	43
Isolates of *R. solanacearum* obtained (no.)	73
Recommended management practices for reducing *moko* in banana (percentage of use by farmers)	
Treating soil with disinfectant	52
Treating tools with disinfectant	90
Exclusive use of tools for infected plants	57
Few changes in harvest personnel	29
Farmers who apply controls recommended by ICA	86

Developing and validating environmentally sound alternatives for the integrated management of moko (*Ralstonia solanacearum*) of plantain (*Musa* sp.) in Colombia

Introduction

Plantain is a staple food in Colombia. Exports although low at present are potentially an important source of foreign exchange. In Colombia, this crop is grown on 350,000 ha throughout the country. Production exceeds 2.5 million tons per year, with 99% destined for the domestic market and the rest for export. In the major production areas—the Coffee Zone, inter-Andean valleys, Colombian Caribbean Region, and the Eastern Plains—the crop has meant an alternative in the face of serious problems of sustainability with other crops. It generates 100 working days per year per hectare, thus improving the quality of life of the inhabitants of these regions.

The central Coffee Zone of Colombia (Departments of Valle, Risaralda, Quindío, and Caldas) is the biggest plantain producer in the country, with an estimated area of 40,000 ha. In this region, together with other plantain-producing areas, associations of small farmers are interested in linking with the market economy and producing cash crops, including plantains. The importance of plantain in marginal regions (Putumayo, Caquetá, Cauca, Nariño) has risen by 20%-30%, stimulating the regional economy.

Moko or banana bacterial wilt, caused by *Ralstonia solanacearum*, is a devastating disease of plantain. It is found throughout Colombia and cuases and estimated annual loss of about US$5.8 million. The problem is compounded by environmental deterioration caused by indiscriminate use of toxic substances such as formol as control agents.

The only controls that farmers currently use are clean seed and cultural practices that are too short term to be really effective in eradicating or detaining the disease.

Objective

This project aims to reduce the impact of *moko* on the plantain crop by generating alternatives for sustainable management, based on improved knowledge of the causal agent. Specifically, the objectives are to:
Identify the minimum inhibitory concentration of 24 substances for disinfecting working tools and soil contaminated with *R. solanacearum*.

Materials and methods

Liquid disinfectants. The following products were used: formol (37% from drugstore); creolin (Cresovec®), phosphoric acid (field, 35%), phosphoric acid (reagent, 85%), bleach (Patojito® = 5% sodium hypochlorite), paint thinner (paint store), Agrodine® Sl, Electowest, Medellin (unexposed, and exposed

to light for 12 h), yodoforo (unexposed, and exposed to light for 12 h), foamy tincture of iodine, lixiviated rachis of plantain obtained through composting (fresh), Ecolife®, lime juice, liquefied sisal leaf , and macerated lime seeds.

Solid disinfectants. Basamid®; freeze-dried, lixiviated rachis of plantain (obtained through composting); urea; calfos; swinglea (100 g of leaves liquefied in a solution of 1 L of 50% alcohol); sisal rib (in fragments); macerated stalks of pencil plant (plant is stripped); and Kocide 101®

Test tubes. Four mL of nutritive broth were added to the first of 14 tubes for each product. To the other tubes of each series, 2.4 mL of nutritive broth were added. Into the first tube, 0.4 mL of the disinfectant to be analyzed was pipetted. For the solid products, 0.4 g were vortex-mixed and 2 mL transferred from the first to the second tube. This procedure was repeated in the following tubes until no. 12 was reached, when 2 mL were discarded. Tube no. 13 had the culture medium and solution (disinfectant), and tube no. 14 had only the culture medium.

Inoculum. Strain 201 (2) 10^{-2}, isolated from soil infected with *moko*, was grown in TZC medium for 24 h at 35°C. To inoculate the test tubes, a base solution of buffer phosphate was prepared with 34 g of monobasic potassium phosphate in 1 liter of distilled water. Then, a 1.25-mL of the base phosphate buffer solution was added to a liter of distilled water. From this solution, 9 mL were added to the test tubes. With this solution, the inoculum was prepared by suspending the bacterium in the buffer. The concentration was prepared to an absorbance of 0.3 in a spectrophotometer at a wave length of 600 nm. With the Neubauer chamber, 2.1×10^8 bacteria/mL were observed. Each tube was inoculated with 0.1 mL of bacterial suspension.

Incubation and evaluation. The tubes were incubated at 25°C under light during the day. After about 60 h, the tubes were evaluated for turbidity. For each product several tubes were cultured in TZC medium to check that the turbidity was caused by *R. solanacearum*. The chemical tests KOH and oxidase were also used.

Results and discussion

Selection of disinfectants for *Ralstonia solanacearum*, causal agent of *moko* in plantain and banana:

> The most effective were formol, Kocide 101®, phosphoric acid (85%), Ecolife®, Basamid®, and phosphoric acid (35%), taking into account the minimum inhibitory concentration (Table 1).
>
> According to product cost, formol, calfos, phosphoric acid (35%), lime juice, and Kocide 101® were the most effective. Calfos and lime juice are the most ecological options. These disinfectants need to be

evaluated in the greenhouse and field. Yodoforo, heavily used in the field, is effective only at concentrations of 5% or more.

Among the products for future evaluation are the seeds, juice, and peel of grapefruit; salt; Long Life®; Phyton 27® (bactericide and systemic fungicide); swimming pool chlorine (granulated); detergent Fab®; lixiviate of lime; and noni seed.

Table 1. *In vitro* evaluation of 24 substances for their inhibitory action on the bacterium *Ralstonia solanacearum*.

Cons. no.	Product	US$/unit	Minimum inhibitory concentration (%)	Minimum inhibitory concentration (US$/L)
1	Formol	0.54/L	0.117	0.0006
2	Calfos	0.06/50 kg	5.000	0.0029
3	Phosphoric acid (35%)	1.01/L	0.625	0.0063
4	Lime juice	0.18/L	5.000	0.0091
5	Kocide 101®	7.97/kg	0.156	0.0124
6	Paint thinner	0.58/L	2.500	0.0145
7	Phosphoric acid (85%)	10.87/L	0.156	0.0170
8	Urea (46%)	0.18/50 kg	10.000	0.0176
9	Bleach Patojito®	0.27/gallon	10.000	0.0272
10	Ecolife®	29.00/L	0.234	0.0452
11	Basamid®	14.50/kg	0.322	0.0470
12	Creolin	4.08/gallon	1.250	0.0510
13	Agrodine®, exposed to light	9.24/L	1.250	0.1155
14	Yodoforo	10.87/L	5.000	0.5437
15	Yodoforo, exposed to light	10.87/L	5.000	0.5437
16	Lixiviate of plantain, freeze-dried	7.25/100 g	10.000	0.7249
17	Agrodine®	9.24/L	10.000	0.9243
18	Iodine tincture	20.84/120 mL	10.000	2.0841
19	Sisal rib fragments	0.18/kg	No control	-
20	Leaf extract from sisal	0	No control	-
21	Leaf extract from swinglea (prepared in 50% alcohol)	0	No control	-
22	Lixiviate of plantain, fresh	0.36/L	No control	-
23	Pencil plant	0.18/kg	No control	-
24	Lime seeds	7.25/kg	No control	-

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Characterizing the Colombian Population of *Colletotrichum acutatum*, Causal Agent of Citrus Anthracnose, using AFLP

Introduction

Citrus anthracnose, caused by *Colletotrichum acutatum*, is a disease that causes considerable losses in orchards because it affects principally flowers and floral buds, thus directly attacking the trees' production and yield.

Previous studies in the main producing area of western Colombia have confirmed the presence of a complex of two species of the pathogen, according to the ITS region of their rDNA and morphological characteristics of *in vitro* growth. One species has been designated as SGO (for "slow-growing orange" strain). It is similar to *C. acutatum* (according to the ITS region), and is considered as causing economic damage through attacking mainly floral organs. It is also tolerant of benomyl. The second species is denominated as the FGG (for "fast-growing gray" strain) group, and is related to *C. gloeosporioides* in that it is saprophytic, ubiquitous in orchards, and sensitive to benomyl.

The above problems encouraged us to carry out in-depth research to characterize the genomic variation of the *C. acutatum* population. We used AFLPs. The technique provides a series of comparable points related to phenotypic traits on which to standardize and group individuals, and search for population subgroups that are specialized for specific conditions or factors such as area and/or host type.

Methodology

Isolates

For this analysis, CORPOICA-Regional 1 supplied us with isolates of *C. acutatum* from different regions of the country and cultured as monospores on paper (Table 1).

DNA extraction

The isolates were cultured in liquid medium (PDA + lactic acid) to produce mycelia for extraction. After 15 to 20 days, mycelia were harvested, dried, macerated, and DNA extracted according to a modified version of the protocol by Dolli and Dolli. DNA quality was verified in 1% agarose gels and quantity calculated by fluorometry.

AFLP

For our situation, we modified and standardized the protocol given in the AFLP manual by Gibco after case. A 350-ng sample of DNA was digested with two restriction enzymes (*Eco*RI/*Mse*I). The digested fragments were ligated to their respective adapters. Then 5 µL of a dilution at 1:5 of the restriction-ligation reaction was amplified with the primers EO = 5'-AGACTGCGTACCAATTC-3' / MO 5'-GACGATGAGTCCTGAGTAA-3', using the thermocycler PTC100. In consecutive order, selective amplification was carried out on those fragments that presented one or two annexed nucleotides, starting with the 1:5 dilution of the previously amplified product (Gibco-BRL, AFLP Analysis System for Microorganisms).

The amplified products were denatured with 10 µL of loading buffer (xylene cyanol, bromophenol blue, and formamide) at 94°C for 3 min. They were then placed on ice and submitted to electrophoresis in polyacrylamide gel at 6% (w/v) in 0.5X TBE electrophoresis buffer. For this analysis, we used the EAC/MA combination (5'-AGACTGCGTACCAATTC/AC-3' and 5'-GACGATGAGTCCTGAGTAA/A-3', respectively), which, in previous studies, showed polymorphism among a small number of isolates, according to differences in area and different citrus hosts.

Statistical analysis

Based on information resulting from the AFLP technique, we generated a binary matrix of data (presence or absence of bands), which was then analyzed with the NTSYS 2.0 statistical package, to generate a similarity dendrogram, using the Dice coefficient.

Results and discussion

A data matrix was obtained from 100 bands for 43 isolates (Figure 1). The similarity dendrogram generated (Figure 2) excluded those isolates that did not amplify. There was a wide range of variability in the pathogen but no clear clustering of isolates by either area or citrus host.

The dendrogram showed two clusters separated by a similarity coefficient of 0.58 (Figure 2, clusters A and C), where A comprised isolates that came mostly from the coffee-growing region. Moreover, of all the isolates from oranges evaluated in the trial, 71% fell in this cluster; and of the isolates from limes, only 47% fell in this cluster. Two subclusters also separated out at the similarity coefficient of 0.8, notably a set of five isolates (subcluster B) from Valencia oranges.

In contrast, cluster C showed high variability, being comprised of isolates that, in their majority, clustered in groups of two isolates below the similarity coefficient of 0.73. About 29% of the isolates forming cluster C are from oranges and 53% from limes, including three isolates from 'Limón

Pajarito', even though these show considerable variability within the cluster. Isolate 671 from 'Lima Tahití', from Magdalena, is very different from other members of the cluster, clustering only at a similarity coefficient of 0.16.

The foregoing shows that, according to host species, isolates from oranges are less variable than those from limes. There is no consistent clustering by area.

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Table 1. Isolates of the fungus *Colletotrichum acutatum* (causal agent of citrus anthracnose) and their origins, used for populational analysis (see also Figure 1).

Citric line	Isolate	Area	Host species as source[a]
1	73c	Caicedonia	NV
2	552cd	Cundinamarca	NV
3	574cd	Cundinamarca	NV
4	648mg	Magdalena	LT
5	671mg	Magdalena	LT
6	653mg	Magdalena	LT
7	10mz	Manizales	LT
8	212c	Caicedonia	LT
9	264ad	Andalusia	Lp
10	8mz	Manizales	LT
11	18r	Armenia	LT
12	1000		
13	23ad	Andalusia	Lp
14	11c	Caicedonia	LT
15	55p	Pereira	LT
16	71mz	Manizales	NV
17	617mt	Meta	Tg
18	1001		
19	7a	Armenia	LT
20	599mt	Meta	Tg
21	621mt	Meta	Tg
22	560cd	Cundinamarca	NV
23	606mt	Meta	Tg
24	267ad	Andalusia	Lp
25	561cd	Cundinamarca	NV
26	14c	Caicedonia	LT
27	539cd	Cundinamarca	NV
28	570cd	Cundinamarca	NV
29	591mt	Meta	NV
30	567cd	Cundinamarca	NV
31	645mg	Magdalena	LT
32	569cd	Cundinamarca	NV
33	107p	Pereira	LT
34	13ar	Armenia	LT
35	201c	Caicedonia	LT
36	6mz	Manizales	LT
37	106p	Pereira	NV
38	132p	Pereira	NV
39	66ar	Armenia	NV
40	1002		
41	98mz	Manizales	NV

42	53p	Pereira	NV
43	1003		
44	398p	Pereira	NV
45	289p	Pereira	NV
46			Marker

a. NV = Naranja Valencia ; LT = lima tahiti ; Lp = limon pajarito ; Tg = tangelo;

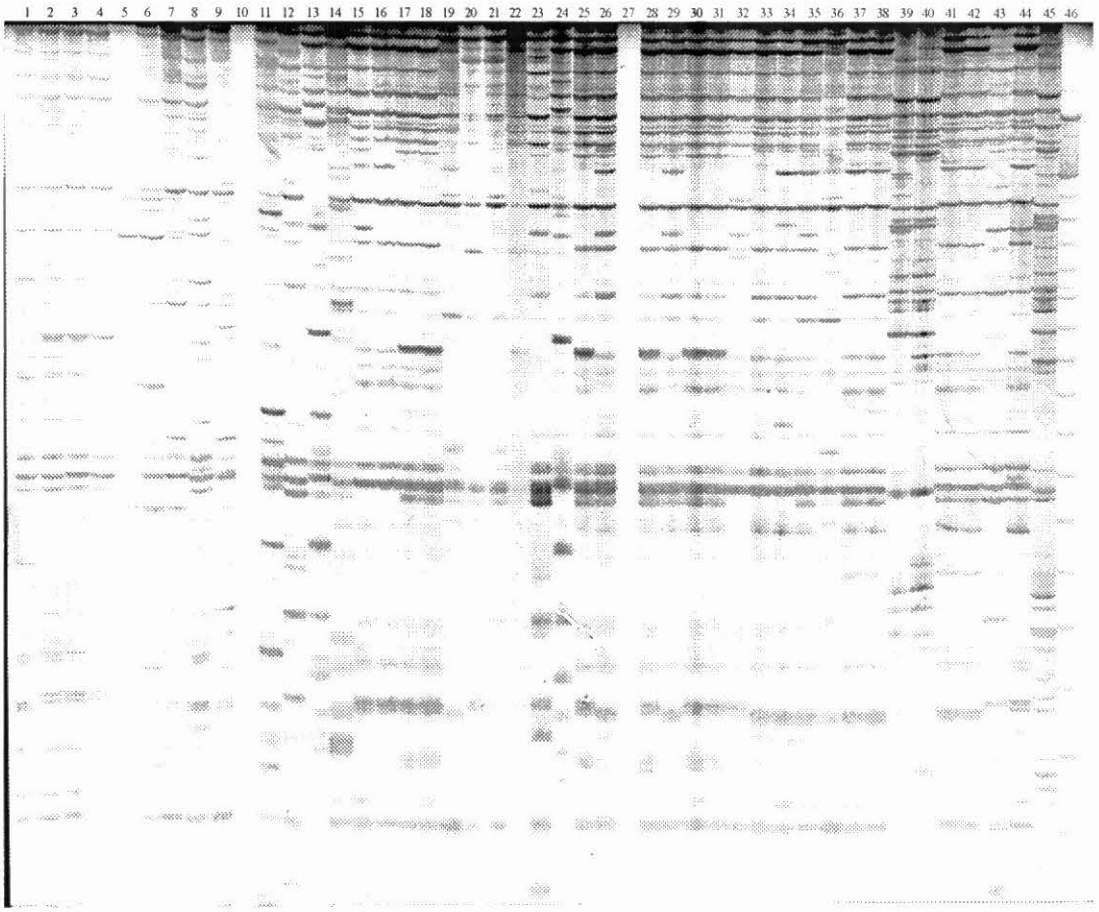

Figure 1. AFLP of isolates of *Colletotrichum acutatum*. Lanes 1 to 45 = isolates of *Colletotrichum acutatum* of different origins; lane 46 = marker, 30-330 in weight.

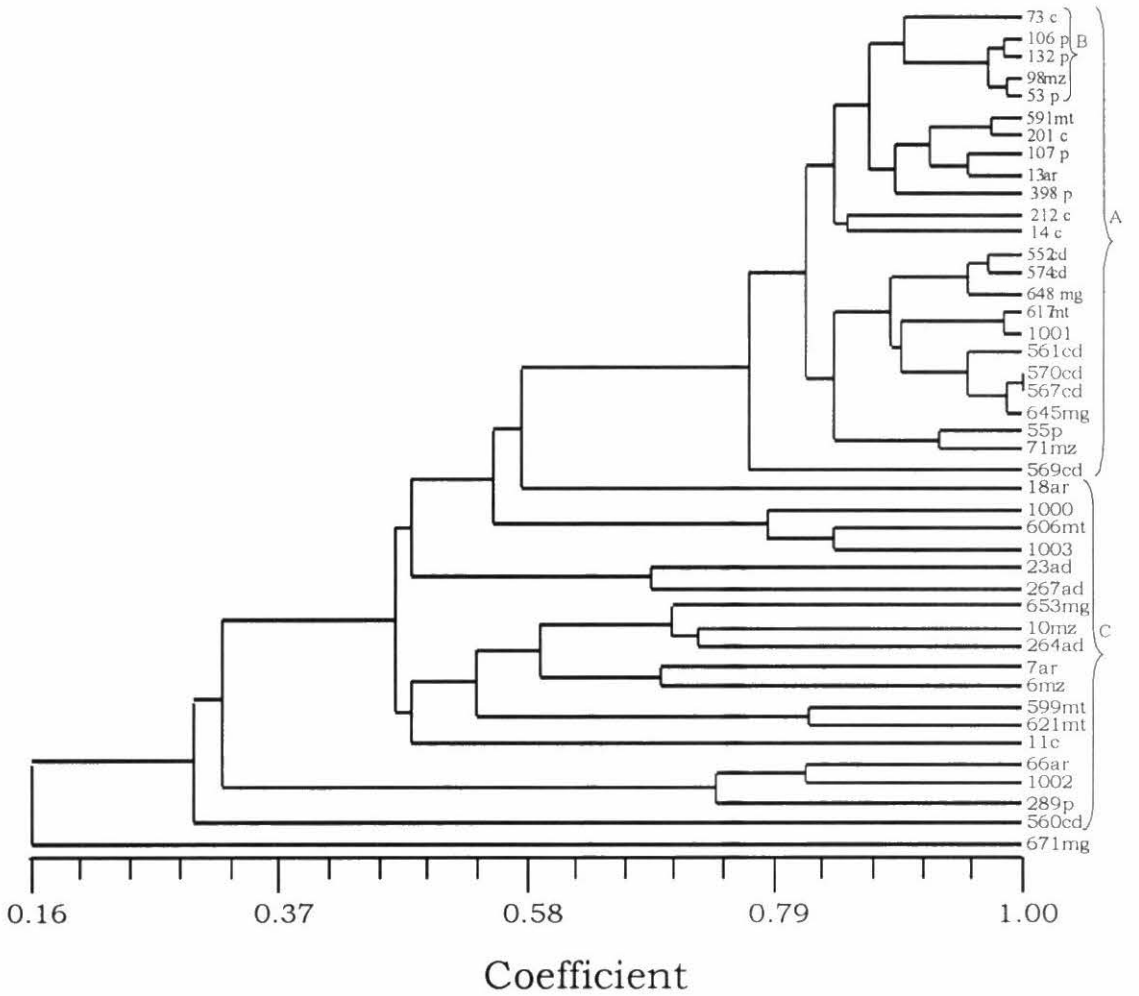

Figure 2. Similarity dendrogram, generated by NTSYS 2.0, for 43 isolates of *Colletotrichum acutatum*, causal agent of citrus anthracnose.