

# IICA

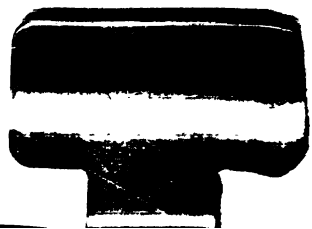


Consultant Final Report  
IICA/EMBRAPA-PROCENSUL II  
PLANT TISSUE CULTURE

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BR-89-  
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**IICA — CIDIA**

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**PLANT TISSUE CULTURE**

**Consultant Final Report**  
**IICA/EMBRAPA-PROCENSUL II**

**Marguerite Quoirin de Ramos**

**Brasília, setembro de 1989**

**INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA**  
**EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA**

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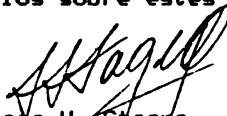
## APRESENTAÇÃO

A reprodução e difusão dos Relatórios de Consultores, no âmbito restrito das Diretorias das Unidades do Sistema Nacional de Pesquisa Agropecuária, vinculado à EMBRAPA, tem como objetivo principal o de divulgar as atividades desenvolvidas pelos consultores e as opiniões e recomendações geradas sobre os problemas de interesse para a pesquisa agropecuária.

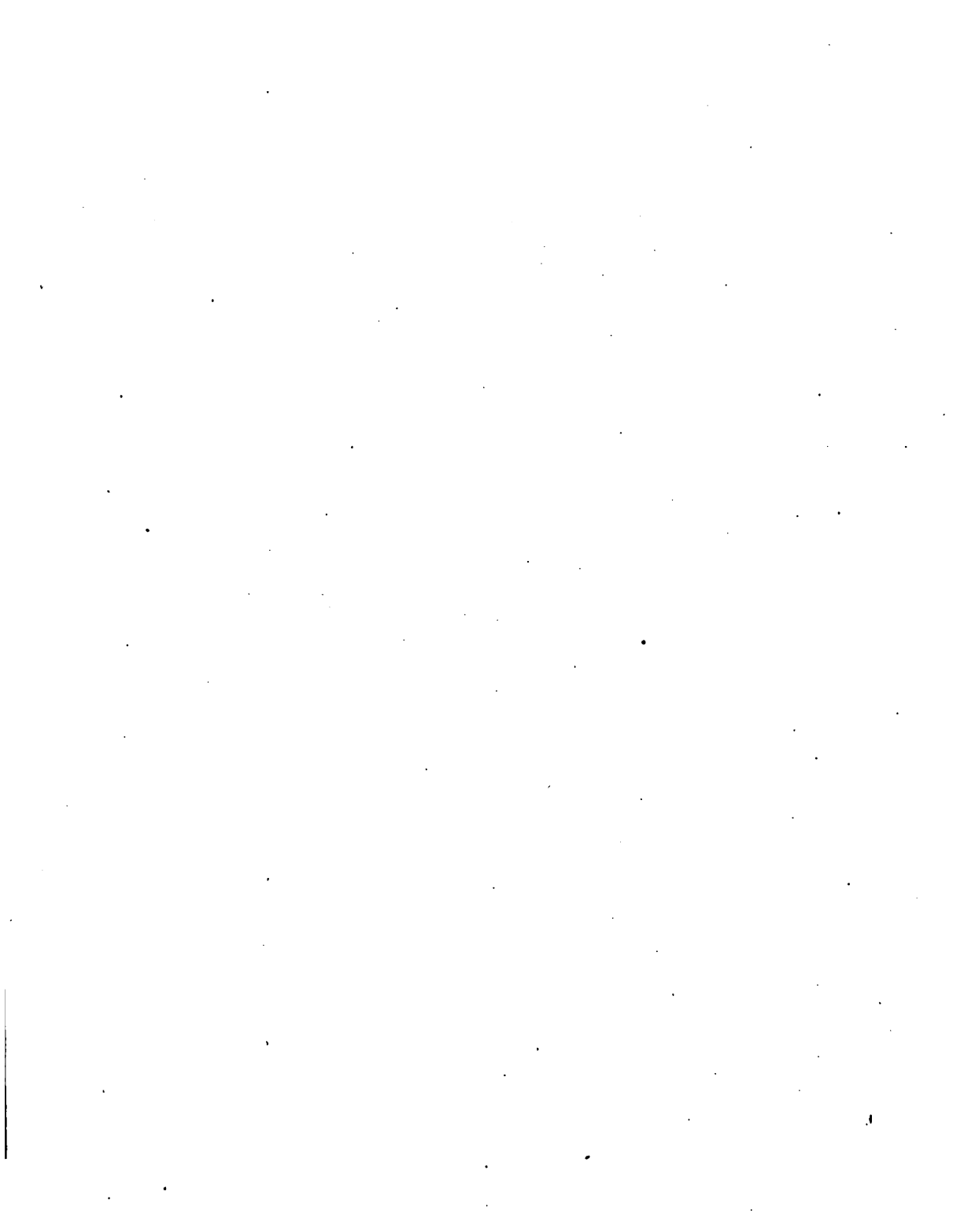
As atividades de consultoria são realizadas no âmbito do Projeto de Desenvolvimento da Pesquisa Agropecuária e Difusão de Tecnologia na Região Centro-Sul do Brasil - PROCENSUL II, financiado parcialmente pelo Banco Interamericano de Desenvolvimento - BID e a EMBRAPA conforme os contratos de Empréstimo 139/IC-BR e 760/SF-BR, assinados em 14 de março de 1985 entre o Governo Brasileiro e o BID.

As opiniões dos consultores são inteiramente pessoais e não refletem, necessariamente, o ponto de vista do IICA ou da EMBRAPA.

A coordenação dos Contratos IICA/EMBRAPA agradecerá receber comentários sobre estes relatórios.



Horacio H. Stagno  
Coordenador Contratos IICA/EMBRAPA





INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE  
IICA/ENBRAPA CONTRACT

CONSULTANT FINAL REPORT

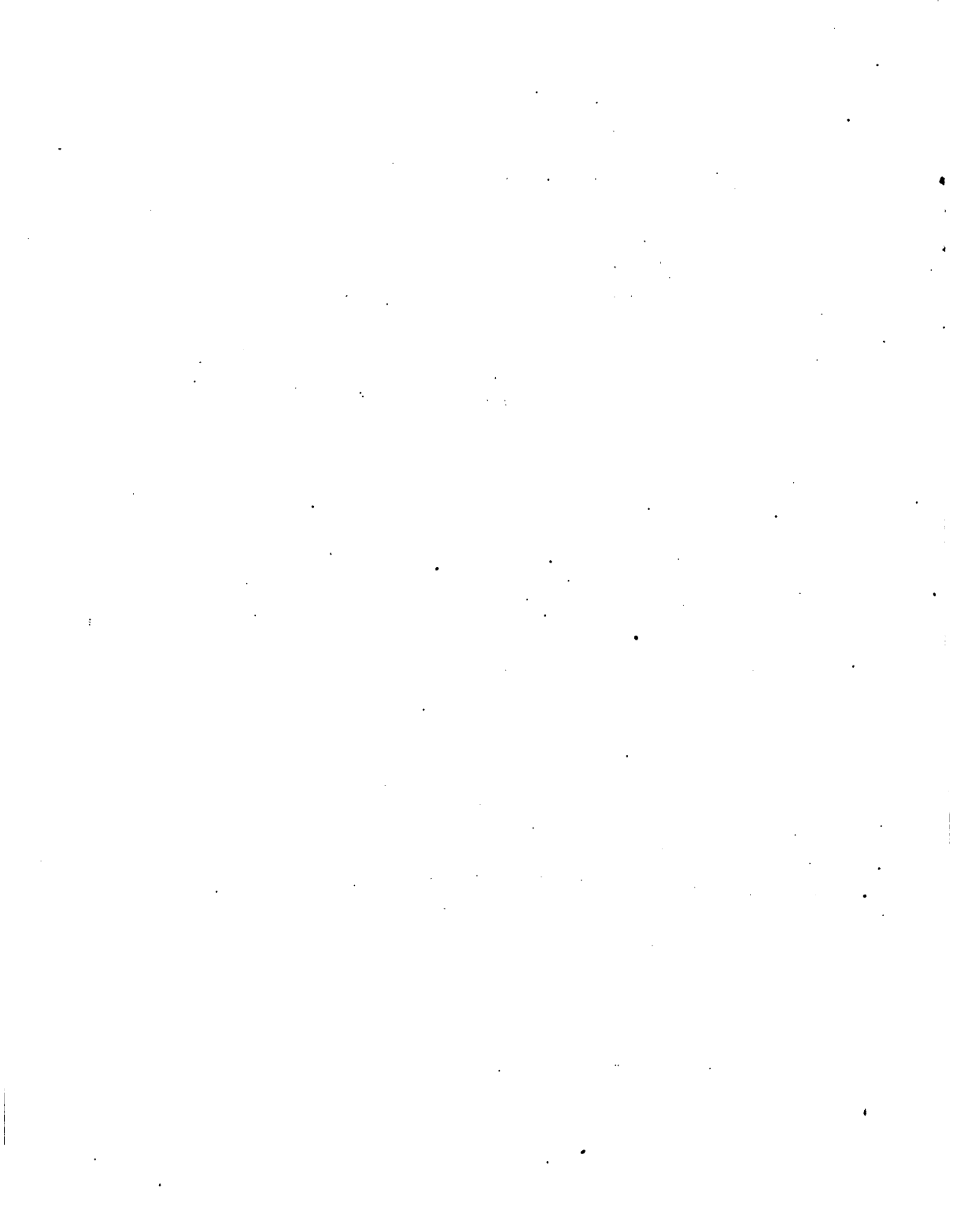
1. Consultant's full name: *Marguerite Quoirim de Ramos*
2. Specialist in: Plant Tissue Culture
3. Title of IICA Project: *2.SB.3.*
4. ENBRAPA Program for which consultancy is provided:

PROGRAMA : *PROCENSUL II*

SUBPROGRAMA : *05-RECURSOS GENETICOS*

IICA Project Activity Code: <i>2.SB.3.05</i>		Administrative Code: <i>R 4894 B1E 03 105</i>	
Title of Activity of IICA Project corresponding to this consultancy		<i>Cooperation with EMBRAPA on research and applications of genetic resources, biotechnology and biologic control of plagues, diseases and weeds.</i>	
CONSULTANT CONTRACT PERIOD		DUTY LOCATION (Center)	
<i>August 31, 1988 to August 31, 1989.</i>		CNPFT - Pelotas	
CONTRACT EXTENTION PERIOD (If any)		DUTY LOCATION (Center)	

5. Financial support: *PROCENSUL II*



## 6. ACTIVITIES UNDERTAKEN BY THE CONSULTANT AND RESULTS

### 6.1 RESEARCH DONE UNDER DIRECT RESPONSIBILITY OF THE CONSULTANT

Research activities developed	Results Achieved
1. Meristem culture and micro-propagation	
1.1. Apple tree	The problem of vitrification was resolved, using a modified multiplication medium with 0.5 mg/l of BAP, 0.5 mg/l of NAA and 3 mg/l of GA <sub>3</sub> .
1.2. Artichoke	The micropropagation process was implemented. The multiplication and rooting conditions were improved in order to get high multiplication and rooting rates.
1.3. Plum tree	The culture conditions allowing "in vitro" development of meristems were studied, but no positive result was obtained till now.
2. Callus formation and plantlet regeneration for somaclonal variation programs	
2.1. Potato cv. Macaca	<ul style="list-style-type: none"><li>- Obtention of callus from stem, tuber and leaf pieces.</li><li>- Plantlet regeneration directly from leaf segments, and from different types of callus. Obtention of 200 plants which will be studied for selection of new traits.</li></ul>
2.2. Sweet potato cvs. Da Costa, Polvilho	<ul style="list-style-type: none"><li>- Formation of callus from stems, roots, leaves and petioles.</li><li>- Proliferation of roots.</li><li>- Obtention of a few shoots regenerated from stem and leaf callus of cv. Da Costa.</li></ul>
2.3. Apple tree cvs. Starkrimson	<ul style="list-style-type: none"><li>- Proliferation of callus and regeneration of adventive shoots from leaves of MM 109.</li><li>- Callus formation from stem and leaf segments of cv. Starkrimson and Maruba.</li><li>- Plantlet regeneration from stem callus of Maruba. The plantlets were grown, multiplied "in vitro" and transferred to soil.</li></ul>
Root stocks { Maruba MM 109	
2.4. Pear tree cvs. Sucre Vert Hood	<ul style="list-style-type: none"><li>- Callus proliferation from stems of both cvs.</li><li>- Shoot regeneration from callus of cv. Sucre Vert. 40 new plants were obtained, grown "in vitro" and then transferred to soil for further studies.</li></ul>

6.1. Research done under direct responsibility of the consultant (cont.)

3. Embryos and cotyledons culture

Apple tree cv. BR1

Pear tree cv. Smith

Peach tree cv. Bolinha

- High Contamination rates in case of apple embryos and peach cotyledons.
- Callus development from cotyledons of apple, pear and peach.
- Shoots development from embryo and cotyledon callus of pear and apple.

4. Potato protoplast isolation and culture

- Some protoplasts were obtained, but could not be maintained in culture.

6.2 SUPPORT TO RESEARCH UNDERTAKEN BY OTHER ENBRAPA RESEARCHERS

Research activities developed	Results achieved
1. Meristem culture and micro-propagation of potato. "In vitro" tuberization. Indexation for virus S, X, Y and leaf roll virus.	Program of pre-basic seeds production (80.000 tubers per year) of the main cultivars produced at the CNPFT.
2. Meristem culture and micro-propagation of apple, pear, peach and plum trees.	Production of virus free material of the main rootstocks and cvs; "in vitro" propagation for research work and distribution to the producers.
3. Meristem culture and micro-propagation of blackberry	Multiplication of new hybrids and cvs. produced by the CNPFT. Increase of multiplication and rooting rates.
4. Asparagus anther culture	Obtention of homozygous (XX and YY) lines for the production of male plants. The work was started in 1988 and is providing good results.
5. Meristem culture and micropropagation of sweet potato and garlic	Production of virus free plants and bulbs in order to constitute basic germplasm for breeding programs. In case of sweet potato, 150.000 virus-free plants were produced and 15 cvs. of garlic were introduced "in vitro".

### 6.3 TRAINING ACTIVITIES DEVELOPED BY THE CONSULTANT

Date	Training subject matter	Type of event*	Number of beneficiaries	
			From ENBRAPA	From other institutions
6.12.89	Production of pathogen free material for fruit growing	Conference	15	35
15.10.89	Meristem culture and micropropagation	Seminar	5	25
28.6.89	Perspective of tissue culture for fruit trees genetic breeding	Conference	25	10
24.8.89	Micropropagation of trees: apple tree and eucalyptus	Conference	0	27

\* Short courses, seminars, conferences, etc.

### 6.4 IN-SERVICE TRAINING PROVIDED BY THE CONSULTANT

In-service training subject matter	Names of counterparts
1. Obtention of somaclonal variants. Implementation of the technique of callus proliferation and shoots regeneration from callus	José Antonio Peters
2. Protoplast isolation.	José Antonio Peters

6.5 ACTIVITIES IN SUPPORT OF RESEARCH STRATEGY AND PLANNING

Research subject matter	Research program to which subject matter is concerned
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Development of research about protoplast culture, somaclonal variation and new techniques of "in vitro" multiplication of horticultural species.

Support to development of the activities of study and applications about genetic resources, biotechnology and biological control of diseases, pests and weeds.

6.6 ACTIVITIES IN SUPPORT OF OTHER CENTERS AND UNIVERSITIES IMPROVING THE RESEARCH CENTERS LINKS WITH ABROAD

Subject matter on which links were recommended	Persons, centers and universities recommended for contact
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Potato breeding by tissue culture for virus and fungi resistance

Dr. P. Lepoivre  
Faculté des Sciences Agronomiques. Gembloux  
Belgium

Somatic embryogenesis in fruit trees (apple and pear tree)

Dr. P. Druart - Dr. P.Boxus.  
Station des Cultures Fruitières et  
Maraichères - Gembloux - Belgium

## 6.7 PUBLICATIONS AND REPORTS UNDERTAKEN WITH THE CONSULTAT'S PARTICIPATION

Author(s)*	Title of publication* or Report and other bibliographic identification
Quoirin, M. and Peters, J.A.	Plantlet regeneration from callus of <u>Solanum tuberosum</u> cv. Macaca
Peters, J.A. and Quoirin, M.	Micropropagation of artichoke ( <u>Cynara scolymus</u> L.)
Quoirin, M. and Peters, J.A.	Plantlet regeneration from stem callus of apple. ( <u>Malus pumila</u> cv. Maruba)
Quoirin, M.	Relatório dos trabalhos de micropropagação e variação somaclonal - 1988/89.

\*The three first papers are in redaction.

\* Personal, institutional, etc.

## 6.8 SUPPORT PROVIDED TO EMBRAPA RESEARCHERS IN THESIS AND DISSERTATION WORK

Name of the student	Thesis subject matter and sinthesis of advice
Daniela Lopes Leite	Micropropagation of pear tree. Type of media to be used for multiplication and rooting. Preparation of plant material, culture media and indications about "in vitro" manipulations.



## 6.9 OTHER ACTIVITIES DEVELOPED BY THE CONSULTANT

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- 1) Support provided to University students:  
U.Fed.Pelotas - Fac. de Agronomia "Eliseu Maciel"  
Master thesis - 27 de janeiro de 1989  
Title of the thesis: "Micropropagação de ameixeira (Prunus salicina Lindl.), cv. Santa Rosa"  
Name of the student: Ariano Martino de Magalhães
- 2) Assistance to congress:
  - International Symposium of Plant Biotechnology.  
ESALQ - Piracicaba - 25-28 october 1988
  - II Reunião Brasileira de Fisiologia Vegetal, Piracicaba - 20-24 february 1989
- 3) Visit of tissue culture laboratories:
  - Univ. Fed. Rio Grande do Sul - Fac. de Agronomia - Porto Alegre, RS
  - Univ. Fed. Rio Grande do Sul - Centro de Biotecnologia - Porto Alegre, RS
  - ESALQ - Univ. São Paulo - Fundação de Estudos Agrários Luiz de Queiroz - Centro de Biotecnologia Agrícola - Piracicaba, SP.
  - Univ. Fed. R.J. Instituto de Biofísica - RJ
  - Univ. Fed. R.J. Instituto de Biologia - RJ
  - Station des Cultures Fruitières et Maraichères - Gembloux - Belgium.
  - Faculté des Sciences Agronomiques - Laboratoire de Pathologie Végétale - Gembloux - Belgium
  - Faculté des Sciences - Laboratoire de Citogénétique - Lonvain - la - Neuve - Belgium.

## 7. OTHER NATIONAL SYSTEM CENTERS, APART FROM DUTY STATION CENTER, ASSISTED BY THE CONSULTANT

Research center	Area of assistance provided by the consultant

## 8. CONSULTANT'S SUGGESTIONS AND TECHNICAL OR INSTITUTIONAL RECOMMENDATIONS FOR THE IMPROVEMENT OF THE RESEARCH SERVICE

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The consultant obtained results which permitted to improve the micropropagation technique of horticultural species, specially in case of apple tree and artichoke. It would be necessary to realize other experiments in order to increase the multiplication and rooting rates of some cvs. of pear tree, and to obtain a better development of plum meristems "in vitro". For pear tree micropropagation, the consultant suggests the use of a two-phases medium, as reported by Viseur (1985), and also different combinations of salts and hormones during multiplication and rooting (for instance medium of Quoirin and Lepoivre, 1977). Plum meristem culture needs to be further experimented, using buds excised at different moments of the year. It is also recommended to test new media, for instance Druart's medium (1987), used for Prunus meristem culture.

The positive results obtained in callus formation and plantlet regeneration of apple tree, potato and sweet potato are very important for the obtention of somaclonal variants. The next step of this research will be the evaluation of these new plants for characteristics of interest, such as pest resistance and adverse climatic conditions tolerance.

On the other hand the morphological and biochemical characters of the new plants must be studied, and the chromosomal analysis realized.

The techniques used must be improved in what concerns the regeneration rates and applied to other cultivars.

Experiments of somatic embryogenesis could be realized with roots of fruit trees, as reported by Druart (1980).

The technique of protoplast obtention and culture must be applied to brazilian cultivars of potato, following the method of Meulemans (1986), once the laboratory dispose of the chemicals and appropriate conditions.

9. AGREEMENTS OR COMMITMENTS ESTABLISHED WITH EMBRAPA RESEARCHERS IN-SERVICE OF  
THE FUTURE DEVELOPMENT OF RESEARCH IN THE CONSULTANT'S FIELD OF SPECIALIZATION

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The research developed by the consultant will be followed by the EMBRAPA counterpart, specially for crops species. On the other hand, a project was established for genetic breeding of potato, in collaboration with the Faculty of Agronomical Sciences of Gembloux (Belgium) and to be supported by the European Economical Community (EEC) (see 6.6.).

In case of fruit trees, a collaboration will be established with the Station des Cultures Fruitières et Maraichères of Gembloux.

10. CONSULTANT'S COMMENTS ON CIRCUMSTANCES WHICH AFFECTED THE CONSULTANCY WORK

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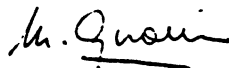
The main conditions of the tissue culture laboratory are very good for work of micropropagation, somaclonal variation and embriogenesis. The personal is competent and the laboratory well equipped.

However, some circumstances did affect the consultancy work. First, in what regards the assays of protoplast isolation, some reactives were missing and could not be supplied rapidly, mainly those who need to be imported (like enzymes). Moreover, the tissue culture laboratory is not totally equipped to realize this kind of investigation (lack of a centrifuge).

Secondly, some problems exist who affect the acclimatation of plantlets in the greenhouse after the "in vitro" culture and induced the loss of some material. The problems are due to the inadequacy of personal in charge of the greenhouse, and inappropriate conditions inside the greenhouse (t° and humidity). This last point can be improved by the supply of some equipment for the control of humidity and temperature. By this way it would be possible to secure a better survival of plantlets in the greenhouse.

Date: September 06, 1989

Signature



## Programa II. Geração e Transferência de Tecnologia

O Programa de Geração e Transferência de Tecnologia é a resposta do IICA a dois aspectos fundamentais: (i) o reconhecimento, por parte dos países e da comunidade técnico-financeira internacional, da importância da tecnologia para o desenvolvimento produtivo do setor agropecuário; (ii) a convicção generalizada de que, para aproveitar plenamente o potencial da ciência e da tecnologia, é necessário que existam infra-estruturas institucionais capazes de desenvolver as respostas tecnológicas adequadas às condições específicas de cada país, bem como um lineamento de políticas que promova e possibilite que tais infra-estruturas sejam incorporadas aos processos produtivos.

Nesse contexto, o Programa II visa a promover e apoiar as ações dos Estados membros destinadas a aprimorar a configuração de suas políticas tecnológicas, fortalecer a organização e administração de seus sistemas de geração e transferência de tecnologia e facilitar a transferência tecnológica internacional. Desse modo será possível fazer melhor aproveitamento de todos os recursos disponíveis e uma contribuição mais eficiente e efetiva para a solução dos problemas tecnológicos da produção agropecuária, num âmbito de igualdade na distribuição dos benefícios e de conservação dos recursos naturais.

## INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA

O Instituto Interamericano de Cooperação para a Agricultura (IICA) é o organismo especializado em agricultura do Sistema Interamericano. Suas origens datam de 7 outubro de 1942, quando o Conselho Diretor da União Pan-Americana aprovou a criação do Instituto Interamericano de Ciências Agrícolas.

Fundado como uma instituição de pesquisa agrônômica e de ensino, de pós-graduação para os trópicos, o IICA, respondendo às mudanças e novas necessidades do Hemisfério, converteu-se progressivamente em um organismo de cooperação técnica e fortalecimento institucional no campo da agropecuária. Essas transformações foram reconhecidas oficialmente com a ratificação, em 8 de dezembro de 1980, de uma nova convenção, que estabeleceu como fins do IICA estimular, promover e apoiar os laços de cooperação entre seus 31 Estados membros para a obtenção do desenvolvimento agrícola e do bem-estar rural.

Com um mandato amplo e flexível e com uma estrutura que permite a participação direta dos Estados membros na Junta Interamericana de Agricultura e em seu Comitê Executivo, o IICA conta com ampla presença geográfica em todos os países membros para responder a suas necessidades de cooperação técnica.

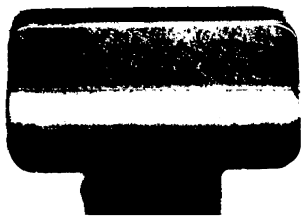
As contribuições dos Estados membros e as relações que o IICA mantém com 12 Países Observadores, e com vários organismos internacionais, lhe permitem canalizar importantes recursos humanos e financeiros em prol do desenvolvimento agrícola do Hemisfério.

O Plano de Médio Prazo 1987-1991, documento normativo que assinala as prioridades do Instituto, enfatiza ações voltadas para a reativação do setor agropecuário como elemento central do crescimento econômico. Em vista disso, o Instituto atribui especial importância ao apoio e promoção de ações tendentes à modernização tecnológica do campo e ao fortalecimento dos processos de integração regional e sub-regional.

Para alcançar tais objetivos o IICA concentra suas atividades em cinco áreas fundamentais, a saber: Análise e Planejamento da Política Agrária; Geração e Transferência de Tecnologia; Organização e Administração para o Desenvolvimento Rural; Comercialização e Agroindústria, e Saúde Animal e Sanidade Vegetal.

Essas áreas de ação expressam, simultaneamente, as necessidades e prioridades determinadas pelos próprios Estados membros e o âmbito de trabalho em que o IICA concentra seus esforços e sua capacidade técnica, tanto sob o ponto de vista de seus recursos humanos e financeiros, como de sua relação com outros organismos internacionais.





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