IICA

Consultant Final Report
IICA/EMBRAPA-PROCENSUL II

BIOTECHNOLOGY AND PLANT BREEDING
WHEAT

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Consultant Final Report
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Emmanuel Piccard

Brasília, janeiro de 1988

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 agradeceria receber comentários sobre estes relatórios.

Horacio H. Stagno
Coordenador Contratos IICA/EMBRAPA

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA
CONVÊNIO IICA/EMBRAPA

RELATÓRIO FINAL DE CONSULTORIA

1. Nome do consultor: EMMANUEL PICARD

2. Especialista em: BIOTECHNOLOGY AND PLANT BREEDING

3. Nome do Projeto do IICA: 2. SB. 3

4. Especificar qual o Programa da EMBRAPA em que a consultoria está sendo prestada:

PROGRAMA : PROCENSUL II

SUB-PROG : II PESQUISA VEGETAL

Código Atividade Projeto IICA: 2.SB.305	Código contábil: R 4874 B1B 03102
Título da Atividade do Projeto do IICA correspondente a esta consultoria	Cooperation with EMBRAPA on research activities in the field of crop production.

PERÍODO DE CONTRATAÇÃO	SEDE DA CONSULTORIA
3 to 20 december 1987	CNPT/EMBRAPA P.Fundo
PERÍODO DE PRORROGAÇÃO	SEDE DA PRORROGAÇÃO

5. Fonte financiadora: PROCENSUL II

Final Report of the Consultancy (2.SB.3) of Dr. Emmanuel Picard under the Responsibility of IICA/Brazil Representative through the IICA/EMBRAPA Contract Coordinator and the Head of CNPT/EMBRAPA.

1. Acknowledgements

The mission has been asked and organized by the Doctor Maria Irene Baggio de Moraes Fernandes who contacted me in March 1987 with the agreement of the direction of the National Center of Wheat Research (CNPT) at Passo Fundo. I am very indebted to the Technical Chief of CNPT, Dr. Aroldo Gallon Linhares, for making exchange and to the Chief of this Center, Dr. Luiz Ricardo Pereira, for the kind arrangements and wellcoming me here. Special recognition is also due to Pedro Paulino Risson who spered no effort for making my life here pleasant and solve administrative problems.

I have also been impressed by the efficiency of IICA and the IICA/EMBRAPA Coordinator in the organization of my consultancy here, sending me the contract, air ticket and providing me for expenses.

But my special acknowledgements are due to Dr. Maria Irene Fernandes and all her team of "Cytogenetics" for receiving me so kindly and for spending the maximum of their time to make my mission the more efficient possible.

This final report has been typed by Dulci Staggemeier to whom I address my sincere thanks.

2. Recall of duties and responsibilities

My consultancy has been centralized around three main points:

"1. To discuss new techniques in biotechnology applied to plant breeding;

2. To review obtained material at CNPT;

3. To participate in technical seminar at CNPT".

(Job description 2.SB.3)

Under these main objectives, a program has been discussed and approved by the Head of CNPT/EMBRAPA for the two weeks spent here, as following:

- Have a deep visit and discussion with Dr. Maria Irene Fernandes on programme of production of doubled haploids of wheat by anther culture and the facilities (laboratory, greenhouse, growth chamber). After that, try to give my own opinion and recommendations.
- Have a series of short individual meetings with the plant breeders to make me understand in a better way the objetives and methods of CNPT/EMBRAPA.
- Give a seminar on the development of my own programme of DH haploids and wheat breeding in France. During the seminar try to open towards new biotechnology for cereals.
- Have a meeting with the Genetic Resources Bank of CNPT.
- Participate to the reinitiation of application of the so called "Bulbosoum technique" to produce doubled haploids of barley in the programme animated by Dr. Gerardo Niclas Árias Duran y Veiga.
- Have an informal meeting with the plant breeders to exchange ideas on the interactions between biotechnologies and plant breeding programmes for mutual interests.
- Give recent information, publications, access to people, concerning recent development of relevant biotechnologies in cereals.
- Participate in a final meeting with the Head of CNPT to give and discuss the main conclusions.

3. Review of the haploid programme at CNPT and recommendations

3.1. General results

As I have been invited in Oct.-Dec. 1979 to initiate at the CNPT/EMBRAPA under the Project UNDP SF 381 (FAO/BRA/69/535) with Dr. Maria Irene Fernandes, here, this programme of production of DH wheat line by in vitro

anther culture, I know it very well. In fact, it is the fourth time that I achieve here a mission in relation with the team of "Cytogenetics and in vitro culture". Therefore, I have been immediately able to communicate with the group and to have a view of the realized progress.

Obtaining doubled haploid lines in wheat has been measured by CNPT as a new interesting technique to obtain quickly homozygosity and make the work of plant breeder easier, especially in the choice of the best lines and to concentrate the effort of the high diversity of problems, encountered here, due to the specific envirnmental conditions in the South of Brazil. The use of DH lines could liberate partially the plant breeder here because this material does not segragate and also all the genes are expressed.

From the beginning of the work (1979) this team has used 201 different genotype coming from different programmes and found that among those 69 were embryogenic lines (35 %). The percentage of embryos related to the number of anthers used ranged 0-10 % and the percentage of embryos regenerating 0-65 %. Up to 9000 spikes were used until to day from which half a million of anthers were plated. A total of about 750 DH were produced, including those of 1987. Some of these DH lines have been tested in the yield trials in this Center and some of them already over yielded the official controls. In the trials conducted in 1986, among the DH lines tested in a block randomized design 8 showed a significant (5 %) superiority compared to the best control with the higher result at 194 % of the control (Maria Irene B. de Moraes Fernandes et al 1987). These very impressive results are promissive and have been obtained with F_1 crosses of selected lines x DH and on the other hand from populations F_2 segregating with an accurate individual plant choice before anther culture, for morphological and adaptative traits by V.R. Caetano. Hence the DH process could have fixed the favorable allelic combinations. Three of these DH lines have been part in the official network of trials of CNPT/EMBRAPA and unfortunately the 1987 results are today, not available. It seems also that these results are really in concordance with those published on Triticale (Charmet and Branlard) or on spring barley (Foroughi Wher et al) which stressed that a possible positive gametic effect could be a relevant explanation of the results.

The team of Maria Irene B. de M. Fernandes has also developed a research

to elucidate the factors which interact with the production of embryos. Unfortunately they did not obtain positive results but rather contradictory ones with the current published work. Nevertheless, it has been proved in one of these experiments that the position of the anther on the medium could be an important factor of sucess in wheat as it has been demonstrated in barley.

In addition, I have observed that this group has produced until 263 DH lines per year which represents a good potential. But during the two last years it is to be denoted that a slight regression in the DH production occurred, due to a series of adverse factors: genotypic effects, bad environment conditons, adverse economic conditions that led to the leaving of a tecnician and many difficulties for the remaining group. These factors have been analysed frankly by Dr. M.I. de Moraes Fernandes with me and seem to be only conjunctural.

The conclusion of this point is that CNPT/EMBRAPA begins to be known as a place where DH technology has been sucessfully carried out. The work of the group has been already published in Brazilian Journal, at a Latin American International Congress and the recent results must be proposed to an International Journal such as Plant Breeding, TAG, especially the field experiments. This last point has been discuseed by Dr. M.I. B. de Moraes Fernandes with me. The suggestion is to demonstrate that DH technology could be a good breeding tool to help the breeders to obtain valuable genetic material in stress conditions. Nevertheless, I have denoted that the results of the group has been already well diffused by Dr. M.I.B.M. Fernandes in numerous semninars, internal meetings and newspapers.

3.2. Special research conducted by the group

I have been impressed by the basic researchs conducted by Dr. M.I.B. M. Fernandes with her students on the realtionship between the pollen polymorphism and the behaviour in vitro. The work has begun one year ago and is realized carefully by Miss Magali Ferrari Grando (Post Graduate Research Stage). The idea is to demonstrate that the diverse percentage of pollen abnormalities encountered in the different tested lines are in good correlation with the results obtained in vitro. In addition this work could

provide a valuable early marker of the androgenetic ability of the genetic material of CNPT/EMBRAPA. During the first part of her stage, Miss Magali Ferrari Grando has denoted and classified the pollen grain around the first pollinic mitosis for eight genotype (DH lines and their crosses). A total of 50.000 pollen grains have been precisely observed and the results high light the enormous diversity of the abnormal pollen grains frequencies within the tested lines and between the same lines. And I suggested that these results are also in correlation with the well known diversity of the results obtained within the same genetic material when tested in vitro. A half day meeting between Miss M. Grando, M.I.B.M. Feranandes and me was devoted to a complete discussion of the results, some propositions for further analysis and for other experiments to be done. My suggestion was to develop a new statistical design and to add early observations in vitro (after 15 days of in vitro culture). After that, if the results are conclusive it is strongly suggested to publish the work internationally.

3.3. Techniques and main recommendations

Dayly visits of the greenhouse, screenhouse, growth chamber, in vitro lab, cytogenetic lab, have been done with the technicians of the group, Gelsi Galon and Clarício Santos, to discuss the different steps of the process of anther culture: mother plants, harvesting the spikes, colchicin treatments, chromosome counting etc.... These visits and another half day meeting with all the group conducted to the following list of recommendations which could be relevant to increase the efficiency of DH production:

3.3.1. Greenhouse equipment

It seemed to me that the greenhouse suffers of lack of equipment, this is a limitation which could have depressed the results. More precisely the optimal light intensity and the more favourable conditions during the growth of the mother plants are absolutely necessary to obtain the better results. In the region, there are long periods with very low day light intensities due to cloudy wheather and the artificial light supply seemed very precarious. I proposed to increase the efficiency of the greenhouse (cooling, lamps ....) and this point has been discussed valuably with Pedro Risson.

3.3.2. Pretreatments

The group of Dr. M.I.B. M. Fernandes has adopted from the beginning of the work, because of the lack of well equiped cold-room (3°C), a kind of post treatment after the anther plating. It seems to me that the efficiency could be significantly increased if the team buys a cold chamber where the spikes will be stocked in dark at 3°C, in tap water only before anther culture. I have seen that the future lab will be fortunately equiped with such a room.

3.3.3. Accurate choice of spikes

I brought with me a recent experiment conducted in France of DH lines produced in my laboratory and with which we carried out again anther culture in my lab and another one. The second one, conduceted by Pierre Deveaux (Firm Florimond DESPREZ) obtained much better results than mine mainly because they are not in "routine production", and, for that, they follow always some recommendations that we delivered ten years ago Dr. de Breyser and me during treining sessions. We said that the spikes have to be very carefully chosen just when we dissect them.

Now we know that colour of the last internode, some phenotypic markers and the colors of the anther are important characters to keep or discard the spikes.

3.3.4. Use of a constant control

The external conditions are so unstable that the results in vitro follow the same unstability. It is absolutely necessary to have a reference to compare the different series. The group agreed with this idea and proposed one of the DH line as control.

3.3.5. Mediums and culture conditions

We reviewed carefully also the used media, the conditions of anther culture. Some modifications have been proposed for the regeneration medium, the thermoperiodism during anther culture, the use of active charcoal. I brought with me two new processes of anther culture in wheat which could

be tested at CNPT/EMBRAPA: 1) medium C17 from Dr. Wang and 2) process of Zhang. Additionaly a lot of little things have been discussed which will not be listed here.

3.3.6. Cytological observations

I strongly suggested to the team to use a doubtless technique for chromosome counting in order to be sure of the exact chromosome number. The use of Ethanol 70 as long term fixator, the addition of Pectinase treatment between Hydrolysis and Feulgen recoloration and the stage within distillated water before squash in carmino acetic drop may be helpful to determine the euploids and aneuploids plants. This point is important because it has been already observed by Dr. Edar Peixoto Gomes an instability of some of the DH lines produced by the team of Dr. Maria Irene.

4. Meetings with the breeders

I had a series of very pleasant and open discussions with some of the plant breeders of CNPT/EMBRAPA: Dr. Ottoni de Souza Rosa, Dr. Edar Peixoto Gomes, Dr. Gerardo Árias, Dr. Augusto Carlos Baier. They explained to me their programme very kindly from the short term to the long term. They all stressed the very limitative conditions of the region with a high amount of diseases, of thermic stresses, of soil toxicity, of rain falls.

There are two great programmes for wheat: 1) the specific one devoted to the transfer of characters (resistances to diseases) by back crosses into the well adapted lines (Dr. Ottoni de Souza Rosa); 2) the general one animated by Dr. Cantídio Sousa with Dr. Edar Peixoto Gomes and Dr. Francisco Langer which concerns the three regions in Brazil for cereals. The general method used is, after numerous crosses with 2 to 4 parents, a modified bulk method or pedigree method. The lines produced by Dr. Ottoni are injected in the general programme. In some places such as Mato Grosso it seems very difficult to surpass the test locally adapted line. If I had a recommendations to do it should be to try to measure the coefficient of consanguinity of the material that they use. It seems to me that it

is certainly very high.

The programs on barley (Dr. Gerardo Áreas) and on Triticale (Dr. Augus to Baier) are also very interesting. The Triticale seems to be very efficient in this stress condition and overyields frequently wheat lines. A programme of crosses primary Triticale 8x x Triticale 6x adapted or primary Triticale 8x x Wheat lines (6x) to enlarge the genetic variability is promissing. These crosses would be worked by anther culture as Dr. Baier proposed.

In barley Dr. Gerardo Árais developed breeding methods involving the evaluations of harvest indices. A better repartition in favour of the kernels would certainly quickly give the better lines. Dr. Gerardo Árias decided also to introduce the DH procedure in these programmes, available in barley with the "Bulbosum technique" as a tool to select the best crosses, to have a more precise measure of the agronomic traits and to begin recurrent selection. During this mission the first embryos obtained by the "Bulbosum technique" have been cultivated in the regeneration medium adapted for wheat and the first plantlet already appeared. This work will be developed in correlation with the team of Dr. M.I.B.M. Fernandes for the in vitro part of the work.

5. The meeting between all the breeders on the DH technology

This meeting has been proposed by Dr. M.I.B.M. Fernandes and has been well attended by all the breeders. Dr. Aroldo Linhares, Technical Chief, was here also. Dr. M.I.B.M. Fernandes gave a short summary of her work, of the main advantages of DH and proposed to the breeders an annual capacity of anther culture. After that the discussions were very open and interesting. The main points discussed were:

1. The annual capacity (winter and summer seasons) of the team of M.I.B.M. Fernandes is 2.000 spikes. If 20 well chosen spikes are used by genotype to obtain DH and evaluate the androgenetic ability, this gives the opportunity for 100 genotypes per year. The proposition is therefore very clear for the plant breeders.

EMBRAPA

2) The stage to which it is interesting to apply the DH technology has been well discuseed: F_1 or F_3 lines coming from the best F_2.

3) The possibility to select the more androgenetic lines and to intercross them to obtain a progress in the production of DH lines.

4) The gametoclonal variation as an implementary efficient mode of action of the DH technology.

5) The possible positive selective effect on the genotype of the microspores through in vitro procedures (gametic selection), in order to obtain the good performances of the DH lines.

6) The introduction of DH lines in a process of recurrent selection.

This meeting seems for me to have been an important moment of this mission and very promissing for the future.

6. Active Germplasm Bank

Dr. Ana Christina albuquerque Zanatta explained to me very kindly her work on germplasm at CNPT/Passo Fundo. One of the main problems is the frequent instabilities of the registered material when she tries to describe it. The concept of pure line is very far from the reality at Passo Fundo. I proposed to her to begin a programme of intercrosses to obtain a genetically large population of introduction of new germplasm within a brazilian genetic background. This idea was agreed by Dr. A.C. A. Zanatta. We decided to reactive the exchange of genetic material between the two laboratories.

Esta publicação foi reproduzida na Gráfica do Escritório do IICA no Brasil, em Brasília, em janeiro de 1988, numa tiragem de 200 exemplares.

Responsáveis pela reprodução: Jadir José dos Santos e Murillo Sodré da Silva.

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 agronômica 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 dc 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.

Interessados em receber mais exemplares deste e de outros Relatórios de Consultores poderão solicitá-los a:

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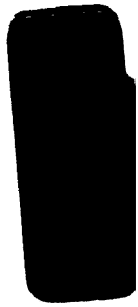

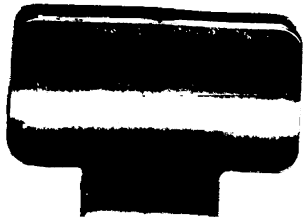

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