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CONSULTANT REPORT

On the National Facility for Plant Tissue Culture
Repository of the National Bureau of Plant Genetic Resources
New Delhi

November 23 - December 3, 1988

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CONSULTANT REPORT

ON THE REVIEW OF THE NATIONAL FACILITY FOR PLANT TISSUE CULTURE REPOSITORY OF THE NATIONAL BUREAU OF PLANT GENETIC RESOURCES NEW DELHI

November 23, to December 3,, 1988

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NEW DELHI: December 3, 1988

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It is a pleasure to acknowledge the cooperation and assistance provided me during the Review. The information received in writing and during discussions was excellent. There has been ample opportunity to meet with staff of the NBPGR and NFPTCR.

I extend special appreciation to Drs. R.K Arora and K.P.S. Chander for coordinating the review and consistently being very helpful in producing the details of information for the review. A special thanks to members of the NFPTCR staff with whom I had extensive discussions.

I am grateful to the Winrock International Institute and particularly Dr. Guy B. Baird, New Delhi, Dr. Floyd J. Williams, Washington D.C. and Mrs. S.N. Bhatt, Washington D.C. as well as Dr. Mark A. Smith, USAID/New Delhi for the opportunity to provide the consultancy service and for the associated financial support.

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Appendix 1. List of persons met or interviewed.

Conclusions and Recommendations

In the two weeks of this review, I have had an excellent opportunity to become familiar with and study the program and activities of NFPTCR. The information received has enabled me to understand the potentials, the quality and accomplishments of the programs to be able to analyze and evaluate for the purpose of making recommendations.

In the following are listed recommendations on the programs, operations and strategies. Every effort was made to include as many topics as possible of those stated in the terms of reference for the review. The comments, observations, opinions and recommendations expressed in this review are intended to be professionally helpful and constructive. They are entirely my own and for which I accept full responsibility.

Recognition is made of the fact that the Ministry of Science and Technology, Department of Biotechnology, had the foresight to provide the generous financial support of this program. In order to continue to achieve and maintain a lead in biotechnology applied to germplasm preservation, it is essential that adequate support be continued in lines with the research which will continue to make the Project a center of excellence.

The impressive, successful results to date and the creation of the project are in charge part due to the initiation, innovation, resourcefulness and hard work of the leadership at NBPGR. Particular credit is due to Dr. Chander for his untiring efforts and capable leadership. The name of the Division, NFPTCR, is unnecessarily long. I recommend that the word "Facility" be deleted. The name of the Division will then be: The National Plant Tissue Culture Repository (NPTCR).

Research and development, program planning and activities

1. The programs on in vitro method for preservation with respect to crop species are well chosen i.e. tuber, bulb crops, fruits, spices are well chosen. The present tissue culture system of shoot tip culture should be compared with an alternate approach. When a procedure is available the method of somatic embryogenesis should be tested and compared with results from the shoot tip procedures. That applies to both culturing and mode of preservation.

2. In the program on non-cultivated, endangered species, a criterion and priority system should be set up and approved. That would ensure that oversight is minimum and that there is an accountability as to what has been selected and why.

3. Preservation method. Attempt should be made to use cryopreservation methods for preservation. The low temperature culture procedure is a successful approach, but the material lasts for a limited time only, more space is required. All of these require labor and time. Somatic embryos lend themselves well to cryopreservation procedures.

4. Genetic stability evaluation. Of the methods now in use, the isoenzyme procedure should be used extensively. Chromosome banding probably is of limited value. Plans should be initiated to develop and implement the RFLP procedures. That would become a separate project. Performing chromosome analysis should also be part of the evaluation.

5. Pollen culture. The present program on pollen culture should be continued and expanded. However, before any research begins a written plan should be prepared. The plan should be developed with the participation of one or more of the plant breeding staff of the respective cultivated and related wild species. Such "team work" will ensure that the choice of pollen sources is correct, that tests will be performed in the form of crosses to determine the suitability of the procedures. The discussed team approach does not exclude the collection and preservation of spores of wild species and their preservation to prevent extinction. For a technical and professional consultant I recommend Dr. L.E. Towill, Colorado State University, Horticulture Department. Fort Collins, Colorado.

6. Protoplasts. Protoplasts can be isolated from the sources of species. There is no justification for cryopreservation, which at best is very difficult and risky.

7. Wide crosses and progeny. The Project should be prepared to undertake preservation of new intergeneric and interspecies crosses. The crosses involve mostly a cultivated species and a wild species or two cultivated genera. The research should be set up as a team approach and involve the breeders and geneticists who have the materials. Similarly, any hybrids developed by protoplast fusion hybridization should also be preserved when plants have been obtained.

8. Selecting species for preservation in vitro. The number of species which can be preserved by an in vitro procedure is large and is likely to increase. To set priorities for choosing species, the relative economic importance to India should be a factor to consider. Other factors are whether germplasm already exists outside India for that particular species, such as the CIAT, ICRISAT Centers. An important factor is also whether a technology exists for in vitro culture.

9. Alternative in vitro systems. The predominate system in use is the shoot tip procedure, and that is very successful on the species of the programs.

   The somatic embryogenesis culture system should be incorporated and possibly compared in initial programs with the shoot tip approach. Somatic embryogenic callus appear to be amenable to cryopreservation, is a ready source of large number of plants as needed and the plants regenerated from such cultures tend to show less genetic instability. The E-callus or E-suspension approach should be investigated when technologies are available for the particular species chosen for preservation.

Biotechnology Programs

1. Antisera for use in viral diagnostics. The NBPGR should take steps to plan for and implement programs in biotechnology. Plant viruses and viroids cause major damage in fruits, horticultural and other economic crops in India. Viruses can be detected serologically or by nucleic acid hybridization probes. Viroids which lack proteins can be detected only by hybridization probes. A comprehensive and detailed plan should be prepared for developing and implementing the antisera procedure for use as diagnostic methods to verify that materials are free of virus. At a later stage similar plans should be developed for implementing molecular procedures used in viroid detection. It is desirable that the program be set up and advantage be taken of collaborative programs with centers and scientists with ongoing programs in immunology and those already having programs involving plant virus pathogens.

2. Plant Molecular Biology. Plans should be initiated to formulate a plant molecular biology program. The activities should be directed at applications in evaluating genetic stability of in vitro generated germplasm. The program should focus on the development and implementation of Restriction Fragment Length Polymorphism (RFLP). Phase one should be the development of a plan and proposal for strategies and applications. Full advantage should be taken of collaboration and interaction with center in India and overseas who have ongoing programs. Such interactions are essential.

Facilities, operation and management

Facilities

If larger scale productions of virus-free plant sticks is planned, there will be a need for a facility which is insect proof. Field testing of in vitro generated germplasm is best performed as a collaboration with breeders, economists and others who are working with the particular crop. The field testing is best done at a center with the climatic conditions suitable for the crop. The proposed equipment for the in vitro facilities is nearly adequate. I strongly recommend that provisions be made to ensure continuous power and a constant water supply. An emergency power supply should be installed. On the equipment I recommend that consideration be given to have an automatic dishwasher, if cost of hand labor becomes uneconomical or less efficient. The same applies to the acquisition of a culture medium dispenser.

Information storage

Data from all programs and records of experiments should be entered into computer storage. The data are then used in evaluation and in future planning . Programs should be acquired to facilitate the development of systems for data storage and statistical analysis.

Management and personnel

Overall the program is managed and operated extremely competently. The research and development achievements are good. However, I recommend that greater use be made of preparing detailed plans for programs prior to starting any experimentations . In addition to objectives, the plan should include strategies, materials and have defined goals to provide end points.

It is important that staff have adequate opportunity to keep abreast of new advances in technology and information. For senior personnel and leaders there needs to be provision for visits and study tours to national and overseas centers with relevant progressive programs. As new technologies and skills are needed, the personnel need to have access to acquire skills at national and international institutions by participation in training courses or short courses.

Communication and collaboration

Exchange of information and interaction with universities and other institutions is very important. Prospects for collaboration in areas of mutual interests should be explored. Steps should be taken to have graduate students participate at NFPTCR in research which are part of the ongoing programs. The research can become the thesis project of the student. The graduate student committee would consist of the academic supervisor and the respective NFPTCR program leader. Such collaboration would speed up progress and be an excellent opportunity for the graduate students. It is also a means of evaluating prospects for new staff.

Transfer of technology

The NFPTCR will acquire expertise and technologies which ought to be transferred to individuals and/or centers which have a need. Plans should be made to establish training courses in the technologies used in the programs. T%he project also should organize and test workshops to promote interaction with agricultural centers, industry and universities.

Future developments

While ICAR may at some point provide the principal funding for the in vitro germplasm preservation project, a biotechnology program, which is highly justified, should continue to be supported by the Department of Biotechnology (MST). The principal programs would be that of the molecular biology.

Consultants

The project should utilize consultants with particular expertise and extensive demonstrated experience and practical expertise in specialized areas of the project.

INTRODUCTION

India is a vast resource of germplasm of agricultural and horticultural crops. Of the estimated 1500 recorded species, a total of 325 are considered to be related to crop species and are potential resources for use in the continuing program of crop improvement. An estimated 160 species are under cultivation in different regions of India. They are crops of agriculture and horticulture with industrial and overall economic importance for India. The crops include major cereals, pulses, oilseed, fruits and vegetables and several plantation crops. India has taken steps to establish a national germplasm repository. The NBPGR has been assigned the responsibility of collecting, evaluating, and conserving the diversity of agronomic and horticultural genetic resources, including medicinal species as well as flavors and spice crops. The materials have been deposited using relatively conventional procedures to maintain the stocks. The tissue culture technologies, as well as the use of liquid nitrogen (cryopreservation) for preserving biological materials, have been used for some time in plant germplasm preservation in different parts of the world. The NBPGR has established a program to use these technologies for the preservation of germplasm. The program is the first of its kind established by a national center of any country. The National Facility for Plant Tissue Culture Repository (NFPTCR) began operation in 1986 and now has research and development in progress. The program objectives focus on species which lend themselves to <u>in vitro</u> technologies, or which are not propagated by seeds, or which are particularly difficult to preserve by more conventional procedures.

OBJECTIVES OF THE REVIEW

The general objectives of the review are to provide scientific and technical advice in the establishment of the National Facility for Plant Tissue Culture Repository.

The specific objectives are:

1. Advise on present infrastructure including possible modification.

2. Appraise relevant ongoing research projects.

3. Provide technical input on freeze preservation of pollen and in vitro cultures in liquid nitrogen.

4. Advise on fingerprinting of accessories and monitoring genetic stability of in vitro cultures.

5. Engage in discussions of new emerging techniques of in vitro conservation.

6. Advise on systems of documentation for these cultures, freeze preserved pollen, and seeds.

7. Suggest international linkages for exchange of in vitro germplasm.

8. Advise on study visit/training programs of Indian scientists.

9. Recommend consultants for this subproject and prepare a written report.

RESUME OF CONSULTANT

Oluf L. Gamborg, Associate Director and Senior Research Scientist, Tissue Culture for Crops Project (TCCP), Department of Biology, Colorado State University, Fort Collins, CO 80521. He grew up on a farm in Denmark. After arriving in Canada, he obtained a B.Sc. in Agriculture and an M.Sc. in Plant Biochemistry from the University of Alberta. His Ph.D. is in Biochemistry from the University of Saskatchewan. He worked at the National Research Council of Canada Laboratory in Saskatoon for 21 years and established the Plant Tissue Culture/Biotechnology Program. He then was the first Research Director in the founding of the International Plant Research Institute, San Carlos, CA, the first plant biotechnology company in the USA. He was a full-time consultant for one year to the Genentech Co., South San Francisco. In the past four years he has been Associate Director of TCCP, a USAID sponsored program at the Colorado State University. During the past five years he has consulted for several private companies, Government of Thailand, Government of Colombia, SA, and has travelled extensively in South Asia as consultant and participates in international research collaborative programs.

ITINERARY

November 23, 1988, Wednesday. Arrived in New Delhi at 1 a.m. Was met at the airport and transportation provided to the hotel by NBPGR.

November 24, 1988, Thursday. Met at the NBPGR Center with Dr. R.K. Arora and Dr. K.P. Chander. I was briefed by Dr. Chander on the NCPGR and NFPTCR, and documents were provided. Also had tour and discussions with scientists-in-charge of different divisions of NBPGR. Attended seminar by Dr. K. Kartha, Consultant from Canada.

November 25, 1988, Friday. Met at Winrock International with Dr. Guy Baird and was briefed on the condition of the consulting. Met Dr. Mark Smith of the USAID. Met at NBPGR with program leaders in tissue culture of the NFPTCR. Attended seminar by K. Martha.

November 26, 1988, Saturday. Met at NBPGR and presented seminar on Tissue Culture Systems and Genetic Stability in relation to germplasm preservation, and on tissue culture and biotechnology approaches to production of stress tolerant germplasm.

November 27, 1988, Sunday. Reviewed written information made available, and from briefings. Preparation of report.

November 28, 1988, Monday. Met with Dr. Baird at Ashoka Hotel to arrange schedule for presenting the final report. First part of report submitted for typing. Met with Dr. Mark Smith and Dr. B. P. Srivastava at USAID. At NBPGR met with Dr. Chander. Received and discussed information on topics of research, operations and issues regarding NFPTCR. Met with research associates on cryopreservation.

November 29, 1988, Tuesday. Met with research associates, and technical staff of NFPTCR for discussions on current and new approaches. Met with Dr. Arora and discussed training and research prospects at TCCP, CSU. Contacted Winrock and USAID. Writing of Report in progress.

November 30, 1988. Wednesday. Met with research associates of NFPTCR for discussions on programs and infrastructure. Writing reports. Presented seminar for USAID and Winrock International.

December 1, 1988. Thursday. Met Dr. Pawda, Dr. Arora and Dr. Chandel at ICAR offices. Discussions on report with Dr. Chandel. Completing of Report.

December 2, 1988. Friday. Presentation and disussion of final report to Winrock/Usaid and NBPGR.

BACKGROUND INFORMATION

The NBPGR has been in operation for more than 10 years and is administered by the Indian Council of Agricultural Research. The activities are carried out through divisions, which are the following: Germplasm Exchange, Plant Quarantine, Plant Exploration and Collection, Germplasm Evaluation and Conservation.

In addition to the central organization, there are eight regional centers. By 1987 the NBPGR had processed more than 27000 accessions to the gene bank and a total of 13000 seed samples had been provided to organizations in India.

In 1986 the National Facility for Plant Tissue Culture Repository was added as an independent part of the NBPGR. The objectives of the organization are the following:

1. Short-term and long term storage of culture of plants, pollen and seeds of economic importance where conventional methods of storage are unsuccessful or inadequate and culture systems are required for genetic engineering of plant cells and biotechnology.

2. Priority is to be assigned to those plants which originated in Indian gene centers and plants introduced from outside for which India has become a secondary center of diversity, or plants of commercial importance in India, wild and weedy species related to our crop plants and wild types of potential economic importance.

3. NFPTCR would have as its main activities, research and manpower training besides its service role.

4. The tissue culture methods already developed for selected plants will be utilized for preservation. For crops on which no work or only meagre work exists, appropriate methodologies would be developed. Work on cryopreservation and freeze preservation of cell, suspension and <u>in vitro</u> culture would be undertaken.

5. National contacts would be established with institutions and experts for information and materials relating to specific crops. Expertise will be built by study tour and visits of scientists abroad for exposure to established laboratories where such work is going on and through inviting foreign experts to India for specific help.

6. Collaboration for exchange of information and material would be developed with international organizations, institutions and individual scientists with similar objectives.

7. Crops for which large scale facilities for collection and preservation exist will be outside the scope of the National Facility for Plant Tissue Culture Repository.

From these objectives a program was designed and an organizational operation structure was established.

The program consists of research components which are:

a. In vitro conservation
b. Cryopreservation
c. Studies on genetic stability
d. Biosystematics

The NFPTCR currently has facilities which are fully equipped. They will be expanded with a new facility to be ready in 2-3 months and will provide needed space and more adequate overall facilities. The establishment of NFPTCR came about because of particular limitations in the more conventional procedures for germplasm preservation, and because technologies in tissue culture and cryopreservation had been developed for practical applications. The activities were to focus on their application to crops and species when the new technologies would be of particular value to ensure safe preservation of the germplasm.

The accomplishments to date in the relatively short period of 2 years are impressive. The choice of species, problems and of particular technologies are good, and are yielding valuable results. The activities for the moment consist of utilizing technologies which are well tested and are readily applied. In tissue culture that means the use of shoot tips and multiple shoot propagations which is expected to involve the least chance of genetic instability. They also involve isoenzyme analysis for classification purposes and tested methods of cryopreservation. There are considerations to adopt and adapt emerging technologies in several categories of biotechnology. The possible choices for the programs of NFPTCR are discussed and presented under Planning and Program Activities and Recommendations.

Plant Biotechnology

In the context of this review, the term biotechnology includes a variety of procedures and techniques for the manipulation of biological systems and genetic engineering with special reference to plants of agronomic importance and selected wild species. The technologies have been developed for practical applications and implementations in the past 10 to 15 years.

Initially the technologies were considered novelties and in some cases, their uses were extrapolated to goals of fantasy. However, during the past four to six years a wide range of technologies of tissue culture, molecular biology and genetic manipulation have been utilized in one or more crops by public and private sector organizations in different parts of the world.

The overriding advantages in using biotechnology are improved efficiency and time saving. The new genetic engineering technologies have produced transgenic plants which have reached the stage of field testing. Crop plants have been produced with demonstrated field resistance to virus and insect pests as a result of genetic engineering. The advances in the next five to ten years can be expected to expand rapidly into new and wider applications. The skills will be developed for isolating genes which are responsible for specific traits such as resistance to nematodes, fungal and bacterial pathogens, insect pests as well as environmental stresses. When the genes for such traits have been isolated, they can be transferred to any crop variety, in which the appropriate tissue culture procedures have been developed.

The development of the new technology and basic information has been confined largely to countries which are relatively more advanced industrially. The countries which at this time are less developed can now begin to take advantage of these technologies and information. The countries which at this time are less developed have the most to gain from the applications of biotechnology. However, to conserve resources and maximize the benefits it is imperative that careful planning precede the implementation of biotechnology.

Biotechnology is often identified with genetic engineering, which is the manipulation of genes for particular traits or products. However, genetic engineering always requires one or more associated technologies. In plants it is tissue culture. Technologies for plant regeneration have become available for producing plants at high efficiency from cultured cells. The procedures are being implemented increasingly in plant production and improvement programs. The use of the technologies has resulted in more rapid and efficient operations in plant multiplication and are speeding up the process of plant breeding. Moreover, the genetic manipulation procedures are already widening the scope and effectiveness of plant improvement. All of the techniques, when designed properly for identified species and problems, gradually will have a major advantageous economic impact on any crop plant activity and ultimately the farmer. A list of technologies and their uses are listed in the tables below. They represent those which are applied in plant biotechnology for crop improvement and production.

I. **Tissue culture procedures for crop production and improvement**

Technologies	Uses	Crop examples
Meristem culture shoot tip culture	disease free planting stock mass propagation	root, tuber crops vegetatively propagated crops
Embryo culture	embryo rescue in incompatible crosses	examples in many major crop species
Anther, ovary culture to produce haploids	one step method to replace back-crossing in breeding	rice, wheat, corn rapeseed, sugarbeet etc.
Callus and cell culture	production of large numbers of plants, screen, selection in vitro	cereals, sugarcane etc. with new traits

II. **Genetic manipulation by cell fusion hybridization or by transfer of cloned genes.**

Transfer and expression of genes in transgenic plants	Add single traits to elite lines in breeding	Virus resistance insect resistance tomato, soybean, etc.
Fusion of cells using protoplasts	produce hybrids of crops with incompatible species to transfer desired traits	Potato, citrus Brassica spp. etc.

III. **Technologies for diagnostics, gene tagging and genetic stability analysis**

Restriction Fragment Length Polymorphism (RFLP)	Detect genetic variations at molecular (DWA) level. genetic maps	Linkage maps for crops: tomato, potato, corn.
Antisera. monoclonal antibodies	Detection of virus and viroids. Identification	Diagnostic methods to verify absence of virus and viroids

The methods in the first part are primarily tools which have been developed and applied in the plant production and for use in making standard breeding more efficient and less time consuming.

The methods in the second part are techniques which are useful for purposes which cannot be achieved by STANDARD BREEDING ALONE. They broaden the scope of what is possible in plant genetics and breeding. The various types of technologies are at different stages of readiness for use in different crop species.

In the third category of technologies are listed two types of procedures which are applications of molecular biology. The RFLP is recognized as an extremely valuable tool in several areas. It will be indispensable in any plant breeding program and germplasm preservation operations. When fully developed, the method will provide a complete map at the molecular level of the genome. The method will detect variations with high accuracy and specificity at the molecular level.

The antisera procedures are already being applied as routine analyses to identify virus pathogens. The monoclonal antibody procedures are also effectively applied.

The NFPTCR is utilizing some of the technologies, and could take advantage of others.

Priorities and Resources

The plans which have been formulated and the project activities which are in progress are based on priorities which are sound and overall are well chosen. Priorities in choices of crops, technologies and types of problems are made after input from several scientific and administrative levels. The total program is based on high priority areas as recognized by central and regional authorities.

The resources of financial support and manpower are adequate. The funds for new facilities will meet the projected need for the size of the unit which is currently envisioned. The positions of personnel which have been approved meets the current need and will allow for hiring expertise for research areas in the planning stage.

While the program and operation has had a strong and successful start, it is imperative that the momentum in progress be continued. A sy em of continuous, careful planning and regular semi-annual evaluation should be in effect to monitor progress and assess results.

The present personnel are skilled for the particular tasks of the current projects. Needs will arise for the technical staff and program leaders to acquire new skills and technologies. Opportunities and access to such training must be available.

Many of the underlying technologies are within the areas of biotechnology. New progress in technology and in information are being made. It is critical that senior staff has access to visits and study tours to national and international centers as an important step in keeping abreast with, and take advantage of, the most recent advances.

Planning and Program Activities

Introduction

The current programs are generally well chosen and the planning also has been good. The topics of the research are appropriate and address specific problems effectively. The planning together with an excellent technical capability have resulted in successful accomplishment, especially in the area of tissue culture. The chosen topics also lend themselves to support good interaction with the total research program. The logic is very good and the lines of research have potential for being valuable, productive and effective. The following sections are intended to provide a general evaluation, followed by recommendations for future activities and action.

In Vitro Conservation

The current research on the application of tissue culture systems for germplasm preservation focuses on five species/crop categories.

In all of the crops the short tip culture system has been employed and has proven successful. The reproducibility of the method is excellent when used on garlic, tumeric, ginger, musa spp and citrus.

The production of several plants from a single shoot tip is advantageous. However the plants being produced can harbor viral and/or viroid pathogens. The chance of some of the plants being genetic variants also exists.

In reviewing the current research projects and activities there are several areas in which efforts ought to be expanded. One area is the tissue culture system. Consideration should be given to use other culture systems. An example is the development and use of embryogenic callus and/or embryogenic suspension cultures. That is particularly applicable where the necessary technology of E-callus formation and plant regeneration is available. In cases where a technology is not available for the particular species or accession, there may be valid justification for developing the necessary protocol. While the shoot tip method is applicable and effective, the use of an E-callus/E-cell culture may have a chance to become more efficient and cost effective.

Some of the advantages include the fact that the plants can be expected to be free of pathogens and that a large number of plants can be regenerated from a given quantity of callus. Moreover, the cell line (accession) can be continued after a period of preservation. There are also indications that embryogenic cells withstand cryopreservation conditions better than other types of cells. This property may be related to the higher cytoplasmic density due to storage components.

In the area of diagnostics, the program should take steps now to plan for the use of antisera in evaluating for presence (or absence) of known viral and viroid pathogens. The program should begin to plan for and establish the necessary facilities to use the antibody technology whenever such sera are obtainable. While consideration can be given to have a basic research project in this area, the highest priority should be given to apply the technology as a means to ensure the health (absence of known viral, viroid pathogens) of the plant materials being preserved (by very costly procedures) and being distributed nationally and internationally.

Although the current plans have not included molecular biology, steps should be taken to prepare plans for and incorporate selected technologies. The use of restriction fragment length polymorphism (RFLP) is a powerful tool for fingerprinting and detection of genetic stability. By means of RFLP it is possible to detect variations at the molecular level. The method is very accurate and precise and is being developed for use in crops species. For the moment the NFPTCR program should prepare to capitalize as much as possible on the existing technology and "clone-banks" as a phase I project. Plans could begin on Phase II, which would involve the development of new RFLR clone banks for species of particular value to the Indian Project. It is imperative that a program to set up and implement molecular biology be planned with extreme care. Senior staff must have opportunity to visit and study research programs overseas, which are using the RFLP approach.

Monitoring genetic stability and ensuring that preserved germplasms yield plants which are true to type is paramount. Systematic and adequate scale (statistical) experiments are necessary to perform. They should cover the species, accessions and each tissue culture system being used. Such research could ideally be performed in collaborative projects with scientists and graduate students of universities. The research would involve program leaders from NFPTCR, a graduate student supervision and a graduate student. The student would undertake the research which would become part of or entirely the thesis research. The research would involve chromosome analysis, phenotypic evaluations, and other trait compositions with the original (if applicable). An associated or separate program could evaluate the effects of the storage conditions and treatments.

In the section below follow on evaluation of the current programs.

Program 1. Root, Tuber and Bulb Crops

The tissue culture methodology for garlic (allium saterum) involving a shoot tip system, which produces 4-6 shoots (plants) is effective and reproducible. The protocol has been applied to about two dozen accessions very successfully. The shoots from the shoot tip culture system have been preserved at 10 degrees centigrade for 1 year with a 40-60% recovery.

There are several areas which ought to be addressed. The research should be performed only on plant materials which have been demonstrated to be free of known virus, nematodes or other pathogens. The plants obtained after the present procedures, which are costly and labor intensive, would not be of any value. Somatic embryogens have been reported for some garlic varieties. Attempts should be made to determine if E-callus production is feasible with the Indian accessions. Plants from such callus lines might be expected to be free of pathogens. There are reports that clovelets also can be produced on callus. The method is not publicly available. The E-callus could be expected to tolerate cryopreservation. Using the E-callus approach should be attempted on an experimental basis with a few accessions to evaluate efficiency, relative costs and advantages over existing methods.

Program 2. Fruit Crops

I. Musa species

The methods in use for banana plantation etc are clearly reproducible and as efficient as the culture system permits. There are now reports on producing plants of the musa spp by somatic embryogenesis. The method should be explored and evaluated for accessions of each species. The use of E-callus could be an advantage.

There is increasing evidence that plants regenerated by somatic embryogenes show exceptional genomic stability (normal ploidy) as compared with those regenerated through organogenesis and by a shoot tip/multiple shoot approach. The reasoning is that unless a genome is free of "defects" the embryogenesis process will not go to completion.

While the musa spp can be presented by lower temperature storage, an embroygenic callus may be amenable to cryopreservation.

II. Citrus spp lemon

An effective procedure using nodal cuttings is in use. This species can also be propagated by producing plants directly from roots.

In the case of Citrus spp the plants produce nucellar embryos; somatic embryogenesis has been reported in some species. The same logic and approaches described for musa spp would apply to the Citrus spp.

Program 3. Flavor, spice and aromatic species

The species which comprise flavor and spice crops can easily be classified as the neglected group. Attempts at improving and preserving these species have had very low priority world wide. The plans for the preservation of ginger and tumeric, hopefully, are only the beginning. Any of the spice and flavor crop species accessions of importance to India deserve to be placed on the highest possible priority list for preservation. India could serve a vital role by obtaining accession (germplasm) from other countries such as Indonesia, Sri Lanka (cinnamon), Madagaskar (vanilla), to name a few. The major spice crops in terms of production are black pepper, vanilla and cinnamon. Many "landraces" are in danger of being lost forever, unless steps are taken shortly to preserve them. Spices and flavors are taken for granted. Tissue culture procedures reported for several spice crops should be adapted and utilized.

Program 4. Non-cultivated, endangered species

Some criteria for selection of species in this category should be formulated. One assumption presumably is that the particular species is not producing fertile seeds.

Preservation Methods

The two general methods being used are lower temperature storage and preservation in liquid nitrogen. The storage at temperatures of 10-15°C has been successfully used on different plants. The storage period is several months. This approach is an option. However storage in liquid nitrogen (cryopreservation) is permanent and has several other advantages. If the species could be cultured as embryogenic cells, cryo preservation has a good chance of being applicable with success. Seeds of plants are divided into two types on the basis of cryopreservation potential. Orthodox seeds such as Brassica spp, onion and pearl millet can be stored readily in liquid nitrogen. The other class are recalcitrant. They are high in moisture content and are less amenable to preservation in liquid nitrogen. Samples of that are cocoa, tea, clove and nutmeg. Programmed freezing protocols are required. For permanency the cryopreservation method is by far the most desirable.

The method should be used whenever feasible. Basic research and development of protocols for particular species will continue to be a significant part of the research.

Genetic Stability

An evaluation of the genotypic stability of the plants regenerated from the tissue cultures is being performed. The principal test is isoenzyme analysis. Such analysis are helpful for classification as well as identification. The chromosome banding procedures are probably of more limited value. Major chromosome changes would need to occur before they will be detected in the bonding pattern. A method which is emerging as a powerful tool is the RFLP. The method is based on restriction enzyme fragment variations. This method will enable mapping an entire gene at the molecular level. Any changes at the molecular (DNA) level is thus detectable. Plans should be made to develop the RFLP method for evaluating the genetic stability in the germplasm generated by tissue culture procedures.

Other Tissue Culture Systems

I. Pollen and microspores

Cryopreservation of pollen is being carried out on a modest scale. The materials are pollen of primitive land races of maize and species related to cultivated maize.

The long term storage of pollen should be a regular part of the research and development program. However, before any research begins, a written plan should be prepared. The plan should be developed with the participation of one or more members of the plant breeding staff of the respective species. Such team work approaches will ensure that the choices of pollen sources are correct; that tests will be performed (crosses) to determine suitability of the procedures. Several types of viability tests can be performed, but none of them equal their use in fertilization (making crosses). The "team" approach will also be of value in making certain that any appropriate sources are preserved.

The discussed team approach does not exclude the collection of spores of wild species and their preservation.

A valuable recent reference on pollen preservation is the review by L.E. Towill entitled: Low Temperature and Freeze-Vacccum-Drying Preservation of Pollen, published in: Cryopreservation of Plant Cells and Organs. K.K. Kartha, Ed. CRC Press. Boca Raton, FL, 1985.

II. Plant Protoplasts

An extensive technology exists for the isolation, culture and uses of plant protoplasts. There would seem to be no justification for devoting time and resources for long term storage of protoplasts. Since they are usually derived from cell cultures or juvenile tissues, a supply of protoplasts can be prepared at any time when needed. One of the important uses of protoplasts is in the production of somatic hybrids to achieve a wide cross or transfer a cytoplasmically inherited trait. When such hybrids have been produced and hybrid plants are available, it would be very important to place samples of such hybrids in long term storage. The hybrid becomes a rare and valuable germplasm.

III. Wide Crosses and Progeny

Intergeneric and interspecies crosses are being produced. The crosses most often involve cultivated and wild species or cultivated genera. Plans should be made to develop procedures for preserving samples of early progeny of backcrosses. Such research should be set up in a team approach with the breeders and geneticists who are developing the crosses.

The same applies to materials obtained through somatic hybridization by protoplast fusion discussed under Protoplasts.

Selecting Species for Long Term Preservation

The task of developing protocols for long term preservation of species has been assigned to NFPTCP. The decision on which germplasm of cultivated and wild species accession are selected lies with the NBPGR. Together with the Division of NFPTCP a decision is made on which germplasm is to be preserved by cryopreservation and which will be subjected to in vitro methods for conservation.

The number of possible species and accessions which can be preserved to advantage by an in vitro technology is large and may be increasing.

To set priorities, two factors should be considered. One is the relative economic importance to India of the species, and the other is whether or not there is in existence a plant regeneration technology. Another factor which also enters in is the extent to which a germplasm already exists on the particular species at a center within or outside India. Examples are those crops which have been assigned to International Agricultural Research Centers. Sweet potato is now the responsibility of CIP.

Alternate In Vitro Systems and Technologies

The predominant method in use is that of shoot tip culture. The logic is that the plant regeneration procedure is reproducible and the method results in the production of several plants in each cycle. The assumption is also that the genetic stability is good. The method has unquestionably been very successful in the species where it has been used. An alternate system and technology which appears to be amenable to cryopreservation is somatic embryogenesis. The method should be tested as an approach in species where a procedure has been developed. Examples are garlic (Allium sativum) and citrus sps of those currently studied, Embryogenic callus can be derived from embryonic or seedling tissues. A culture should be possible to obtain within 3 months. The plant regeneration protocol, which may need adjustment for different accessions, should be defined before long term preservation is attempted.

The somatic embryogenic approach has the following potential advantages:

a. A larger number of plants can be produced in a shorter time with less labor input.

b. The E-culture can be preserved by cryopreservation.

c. The chances for genetic instability and undesirable variants are lower.

d. If needed, a large population of an accession can be obtained more quickly than by the shoot tip procedure.

It should be pointed out that all of the factors above must be verified for each accession or species. In some cases an embryogenic suspension culture may be possible. However, such cultures require shorter incubation and must be carefully maintained by more frequent subculture than E-callus cultures.

Biotechnology Programs

The NFPTCR should take steps to plan for and implement programs in the category of Biotechnology. The one program will involve the use of antibody procedures to assay for virus pathogens and ensure that a germplasm is virus-free. The other program involves the development and application of molecular biology to assay for genetic stability.

Antibody Production and use in Virology

Plant viruses and some viroids cause major damage in fruit crops, horticulture crops, ornamentals etc. in India. Viruses can be detected serologically or by nucleic acid hybridization probes. Viroids which lack a protein can be detected only by hybridization probes. These procedures should be utilized in the diagnostic assays of NBPGR and especially in the certification of in vitro produced and preserved germplasm. Some preparation is already in progress in the Division of Plant Quarantine at New Delhi. The activities should be expanded. A detailed plan and proposal for an expansion should be prepared and approved prior to proceeding. During planning and continuing beyond, the pathologist(s) in charge should visit locations in the USA, where serum banks are located and these procedures are in use routinely for virus and viroid identification and detection. Discussions should also be initiated with the National Institute of Immunology and Dr. M.D. Mishra, Center for Advanced Studies in Virology at IARI to obtain information and explore possibilities for collaboration in joint use of facilities and exchange of information and technology.

In the first phase the plans should include only the acquisition and use of antisera for virus detection. Attempts should be made to obtain and use the sera for selected virus, which are available. Then proceed stepwise to develop sera perhaps with collaboration for a priority list of virus. Perhaps virus purification facilities of other institutions can be utilized in a collaborative program of mutual interest. The intent then is to use the antisera to diagnose diseases, and screen purportedly virus-free materials of tissue culture. The use of the diagnostic technology for detection of viroids should be a phase 2 program. That will involve the use of molecular probes , and the activities will relate closely to a molecular biology program.

Plant Molecular Biology

Plans should be initiated to formulate a plant molecular biology program. The activities should be directed at applications in evaluating genetic stability of in vitro generated germplasm and possibly the production of germplasm of transgenic plants with new agronomic traits.

The first program should focus on the development and application of Restriction Fragment Length Polymorphism (RFLP). The RLFP is a method which enables detection of genetic variations at the DNA (molecular) level. The variations are observed as differences in length of DNA fragments produced by restriction enzyme digestion. The RFLP method is emerging as a powerful tool in varietal identification and genetic analysis of traits of economic importance. When a genetic map has been developed the method permits tagging of any genes of economic importance and facilitates transfer of the traits between breeding lines.

The method when developed is copied and will identify differences in DNA sequences which are not reflected in observable phenotypic changes.

The method has applications in evaluating genetic stability in in vitro generated germplasm.

The technology is based on the use of standard molecular biological techniques involving DNA probes and screening. Phase one should be the development of a plan and proposal for strategies and applications. It is essential that the scientist(s) in charge have personal contacts by visit and study at centers in USA, where the RFLP methods are in use and the technology is well advanced.

Facilities, Operations and Management

Facilities

The current facilities in operation and those projected are to be completed early in 1989.

According to information available these facilities will adequately accommodate the current programs and others involving biotechnology, which may be added.

In some of the areas of technology there will be an evolutionary progression of needs for additional space. The culture facility will consist of walk-in-growth rooms with environmental control of light and temperature. A number of growth chambers (reach in) should be acquired. They are particularly important during technology development where light and temperature requirements are being defined.

Currently there are greenhouses or enclosed structures for growing of R1 materials. A mist chamber for use in the first period after transplanting of R0 (regenerated) plants is essential.

If larger scale production of virus-free plant stock is planned, insect protected growth facilities are required. Field treating would best be performed as a collaboration with breeders, agronomists etc, who are working with the particular crop. That would presumably be done at a regional center with the appropriate climate for the crop.

Information Storage and Processing

Data will be accumulating at a rapidly increasing rate. All data from in vitro operations, storage operations and any of the evaluations should be entered into computer storage.

Programs should be adopted for the processing and analysis of the data. Such an operation will require one full time person, who will work with each of the program leaders in the NFPTCR project. The information should be set up and accessible to the larger computer facility of NBPGR.

Management and Personnel

The current personnel are capable, skillful and motivated, and take a keen interest in their work. They are to be commended together with management who have created an atmosphere which encourages and recognizes these qualities. A characteristic of the operation is the participation and input by program leaders and technical staff. They constitute ideas to increase efficiency and effectiveness of the work.

A current weakness which can be readily rectified is a lack of detailed planning for specific programs prior to starting experimentation. A plan should state what the objectives are, what strategies will be used, what materials (and why) will be used and what the actual goals are. It is also valuable to include the estimated time requirement.

In order to monitor progress each program should have a report session quarterly to the entire group. Using a rotation system that would mean a meeting twice monthly. At such meetings there would also be discussions on issues of laboratory operations, but not personnel problems.

It is very important that staff have adequate opportunity to keep abreast of new advances in technology and information. For senior personnel and leaders there needs to be provision for visits and study tours to national and overseas centers with relevant progressive programs. As new technologies and skills are needed, the personnel need access to acquire the skills at national or international institutions and participation in training courses or an extended stay at institutions.

Communication and Collaboration

The NBPGR including the NFPTCR has access to library facilities on campus. These facilities should include access to world-wide data bases for agricultural sciences.

It is also vital for communications to involve personal contacts. As an example, a research/program leader using in vitro technology with *Musa* species should have personal contacts with breeders, agronomists, geneticists who are working with improvement/breeding of these species. There should be exchange of information on a regular basis.

Exchange of information and interactions with universities and other institutions are very important. Prospects for collaboration in areas of mutual interests should be explored. Steps should be taken to have graduate students work at NFPTR on a research project which is part of the ongoing program. The research becomes the thesis project of the student. The committee would consist of the academic supervisor and the respective NFPTCR program leader. Such collaboration would speed up progress and be an excellent opportunity for the graduate students. It is also a means of evaluating prospects for new staff.

The NFPTCR can make a valuable contribution to the Plant Tissue Culture Unit of the IBPGR (Dr. L.A. Withers). Regular contacts and exchange of information should be made.

Future Developments

The current project has financial support from two sources. One of them is the Ministry of Science and Technology's Department of Biotechnology. While NFPTCR is, and should always remain, a part of the NBPGR, a basic biotechnology research program could form part of the Project. While ICAR may at some point provide the principal funding for the germplasm preservation aspect, a biotechnology program could be maintained and supported through MST/Biotechnology. An example is the recommended program in molecular biology.

Consultants

The projects should have access to consultants with practical expertise in specialized areas of the project. A list of scientists and institutions will be provided by Dr. Oluf L. Gamburg, the consultant for this review.

Transfer of Technology

The NFPTCR will acquire expertise and technologies which ought to be transferred to individuals and/or centers which have a need. The project will also have extensive facilities.

Plans should be made to consider setting up training courses in the technologies used in the project. The courses should provide some lectures, but primarily result in hands-on technology and skills. The project also should consider organizing workshops and mini-symposia. They should be directed primarily at establishing interaction with plant scientists, breeders, from public and private sector national organizations.

Such events are time consuming if planning is to be good. Restraint should be used in deciding on the frequency. Careful planning is of the utmost importance. The workshop should be a "working" one in which the participants will arrive at recommendations in specified areas of issues.

Appendix 1.

List of Persons met or interviewed during the review.

Dr. R.S. Paroda, Deputy Dir.Gen.(Crops), ICAR
Dr. R.K. Arora, Director, NBPGR
Dr. K.P.S. Chandel, NFPTCR/NBPGR
Dr. Guy B. Baird, Winrock International
Dr. Mark A. Smith, USAID, AR
DR. B.P. Srivastava, USAID, AR
Dr. P.P. Khanna, NBPGR
Dr. B.P. Singh, NBPGR
Dr. J.A. Thomas, NBPGR
Dr. Ram Nath, NBPGR
Dr. Bhag Mal, NBPGR
S.R. Bhat, Tissue Culture
B.B. Mandal, Tissue Culture
Ms Ruchira Pandey, Tissue Culture
Ms Neelam Sharma, Tissue Culture
S.M. Balachandran, Tissue Culture
K.V. Bhat, Genetics
Suman Lakhnpaul, Cryopreservation
E. Roshini Nayar, Biosystematics
S. Rama Rao, Cytology
J.L. Karihaloo, Genetics
Alka Sehgal, Biochemistry
P. Chitralekha, Histology
Rekha Chaudhury, Cryopreservation
Dr. K.K. Kartha, Plant Biotechnology Institute, Saskatoon, Canada