PLANT GERMPLASM CONSERVATION : PERSPECTIVES FOR THE 2000s



Taiwan Agricultural Research Institute
Taichung, Taiwan, Republic of China
1994

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Organizational Structure of the Symposium

Co-sponsors:

Asian Vegetable Research and Development Center (AVRDC)

The Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO)

National Science Council (NSC), The Executive Yuan, R.O.C.

Institute of Botany, Academia Sinica

College of Agriculture, National Taiwan University (NTU)

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PLANT GERMPLASM CONSERVATION : PERSPECTIVES FOR THE 2000s

Proceedings of the international symposium held on August 10 -11, 1993 at the Taiwan Agricultural Research Institute, Taichung, on the occasion of inaugurating the National Plant Genetic Resources Center (NPGRC)

Counseling agencies

Council of Agriculture, The Executive Yuan, R.O.C.
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Executing agency

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Plant Germplasm Conservation: Perspectives for the 2000s



COA chairman Paul M. H. Sun opened the NPGRC inauguration ceremonies.



A tour of the new Center's display area. Shown are E. Bird (left) H. L. Shands, K. W. Riley, E. C. Cocking and C. H. Chou.



Deputy Commissioner W. J. Ho of the PDAF addressed the participants and opened the Symposium.



TARI presented each one of the invited speakers with a plaque of appreciation. Dr. Shands is in this photo.



Dr. H. L. Shands presented to the Center a memento taken from the "Seeds of change" book.



TARI Associate Director C. H. Sean presented advisor T. T. Chang with a plaque of appreciation.

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OPENING ADDRESS AT THE NATIONAL PLANT GENETIC RESOURCES CENTER INAUGURATION

Dr. Paul M. H. Sun, Chairman
Council of Agriculture, The Executive Yuan
Republic of China

Distinguished guests, Commissioner Ho, Director Tu, ladies and gentlemen:

Today we inaugurate the opening of the National Plant Genetic Resources Center. First, allow me to salute the tireless toil of Director Tu and the staff members of the Taiwan Agricultural Research Institute for the past several years in leading to this auspicious occasion. Meanwhile, special thanks are due to Dr. T. T. Chang, who used his unique expertise and rich experience in guiding the planning and execution phases of establishing the Center from the very beginning. Their efforts, together with the help of our government engineers, enabled the smooth implementation of the National Plant Genetic Resources Center (NPGRC) project.

Crop germplasm is a treasure and heritage from Nature. It is also a national and precious treasure that belongs to all people of the land. It provides the basis for accelerated agricultural development. In order for crop breeding to enjoy sustained advances and the continuous improvement of the crop cultivars, and for the uninterrupted rise in product quality and production efficiency in our agriculture, the primary moving force comes from rich and broad-based genetic variability in plant resources.

During the past decades, rapid economic development and dramatic rise in the human population of the world, coupled with the extensive development of natural landscapes for agricultural production and/or industrial use, hitherto balanced ecosystems in various parts of the world have suffered irreparable damage. These led to the disappearance of wild plant species to the brink of extinction. Meanwhile, as the industrialization of agriculture grew, crop cultivars became more uniform, and genetic diversity in indigenous cultivars greatly diminished. Presently, industrialized

nations of the world have markedly expanded their efforts in genetic resources conservation and improved their germplasm preservation facilities, in an attempt to cope with germplasm loss and staving off the threat to agricultural development.

The Council of Agriculture has for some time recognized the conservation of genetic resources as an important facet of long-term and sustained agricultural development of our country. During 1988 a research project to study the plan of establishing a National Plant Genetic Resources Center was initiated. Scientists were despatched to study national plant germplasm systems and their progress in Europe, the U.S., Japan and at IRRI, aiming to establish our national plant germplasm system with Taiwan Agricultural Research Institute serving a central role. Over the years a total of four hundred and sixty million New Taiwan dollars was allocated to support the planning and construction of the facilities at the Center and three outlying field repositories.

This day marks the inauguration of NPGRC. I hope that with this auspicious start, we can catch up with the industrialized countries in strengthening the acquisition and preservation of plant genetic resources, conserving the rich germplasm vital to our agricultural development, and enabling this Center to grow into a gene bank of global importance and fame. Meanwhile, with the establishment of a germplasm information system, plant breeders and crop researchers in our country can have easy access to breeding materials, upgrade progress in breeding and accelerate agricultural development. Moreover, the facilities will conserve the disappearing wild plant species -- measures that will also help rehabitation of the damaged environments.

It is my sincere wish that the local and foreign scientists and specialists present today will make full use of the information and experience presented at this Symposium and transmit them to the Taiwan Agricultural Research Institute, so as to assist the implementation of our crop germplasm system, to reach the projected functional capabilities, and to attain the goal of establishing the National Plant Genetic Resources Center.

Also, my best wishes for you ladies and gentlemen. Good day.

OPENING ADDRESS

Dr. W. J. Ho
Deputy Commissioner
Taiwan Provincial Department of Agriculture and Forestry

Distinguished guests, speakers, participants, ladies and gentlemen:

Good afternoon! It is a good pleasure and real privilege for me to open this Symposium on Plant Germplasm Conservation -- Perspectives for the 2000s. On behalf of Dr. Tjiu, Commissioner of Taiwan Provincial Department of Agriculture and Forestry, I would like to extend our sincere welcome to all the participants, especially those who have come from overseas.

It is well recognized that plant germplasm is one of the crucial components for developing new crops and cultivars in agricultural improvement. The genetic variability in plant germplasm is not only the motivating force of crop improvement programs, but also essential to maintaining balanced ecosystems and supporting sustainable agriculture. Progress in crop breeding depends on rich genetic resources and their exploitation.

Our government is aware of the importance of preserving germplasm, and, therefore, this National Plant Genetic Resources Center was brought into existence. Taiwan Agricultural Research Institute, the place where this Symposium is held, has been chosen to implement the national plant germplasm program and to house the Center. After three years of intensive efforts, the Center is now open for use. The exploration, conservation, and utilization of germplasm are important tasks for this Center.

This Symposium's theme related to the policy, management, research and international collaboration of crop germplasm will receive detailed discussion by various experts. We would like to share your experience for managing the gene bank and further our cooperation with international and other national plant genetic resources centers. To our local participants, it is also a good opportunity for them to exchange information, ideas, and to discuss many problems related to international cooperation. I hope the discussions will be fruitful, the participants will become friends, thus raising the prospects for future cooperation.

Again, my sincere welcome to all the overseas participants. Thank you for your coming. I wish all the participants a very enjoyable and pleasant stay in Taichung area. Thank you very much for your attention.

INTRODUCTORY REMARKS

Dr. Te-Yeh Ku, Director
Agriculture and Food Division
Council of Agriculture
Republic of China

It gives me immense pleasure to participate in the inauguration of the NPGRC and the opening of this international symposium. The symposium not only celebrates the coming into being of a magnificent gene bank but also the beginning of the national plant germplasm system.

Looking toward the future, I would like to suggest a number of areas for future endeavor. First, to couple with the project on surveying Taiwan's native flora and fauna and documenting the resources, full efforts to collect, conserve and document the wild plant species should be executed. Such resources will prove to be useful in the development of our agriculture and in international germplasm exchange.

By exploiting the computer capability in the country, we can build up a world-class information system and network on germplasm management and interchange, enabling us to become an important member in the world community of germplasm centers.

Through the simplification of querying conserved germplasm, we can raise the efficiency in its use and strengthen crop improvement at various research institutes and experiment stations. The benefits can be extended to research and improvement of nursery plants and help the nursery industry.

In addition to routine work on collection, preservation and characterization, research on germplasm preservation technology will be an essential work item. Research ranging from traditional storage methods to *in vitro* techniques and cryogenic approaches will help to raise the efficiency and quality and germplasm preservation.

Lastly, the conservation of diverse plant materials will offer useful materials for research and educational purposes. For instance, a field repository could serve to educate the common people not only on conservation approaches but also recreational opportunities and protection of the

environment.

I trust that active discussions at this symposium will lead to useful guidance to our future efforts.

On behalf of the Council of Agriculture, the Provincial Department of Agriculture and Forestry and other cosponsors, I welcome and thank you for taking part in this symposium. I wish you success in your presentations, discussions and deliberations.

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THE U.S. NATIONAL PLANT GERMPLASM SYSTEM AND FORTHCOMING LEGAL ISSUES ON GERMPLASM RIGHTS

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USDA-ARS, Beltsville, Maryland, USA

The U.S. National Plant Germplasm System

While the U.S. National Plant Gemplasm System (NPGS) has been functioning informally for over 30 years, its role was formally established in 1990 legislation authorizing the National Genetic Resources Program (P. L. 101-628). The U. S. Department of Agriculture's Agricultural Research Service (ARS) is charged with the specific role to acquire, characterize, preserve, document, and distribute germplasm to scientist-users for research and breeding. The NPGS (Figure 1) focuses to serve the user community. Interfacing with the users are the working elements (Table 1) of the system: the service activities, the active collections, and the base collection. The next tier bridges the operations and policy elements of the NPGS and the final tier involves the administrative and financial support elements. In their NPGS administrative roles, in addition to ARS, are other Federal agencies such as the Animal and Plant Health Inspection Service (APHIS), interested scientists and administrators from the State Agricultural Experiment Stations System and from the private sector. Over forty Crop Advisory Committees (CACs) composed of Federal, State, and private sector scientists and administrators representing commodities or commodity groupings help assess and prioritize activities relating ot their specific interests. The focus is on user groups, Particularly the breeders, makes the Crop Advisory Committees vital to thesuccess of the system. All parties provide recommendations on how best to achieve the long-range goals of conservation and utilization.

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[1] Associate Deputy Administrator for Genetic Resources, USDA/ARS/NPS, Beltsville, Maryland 20705, USA.

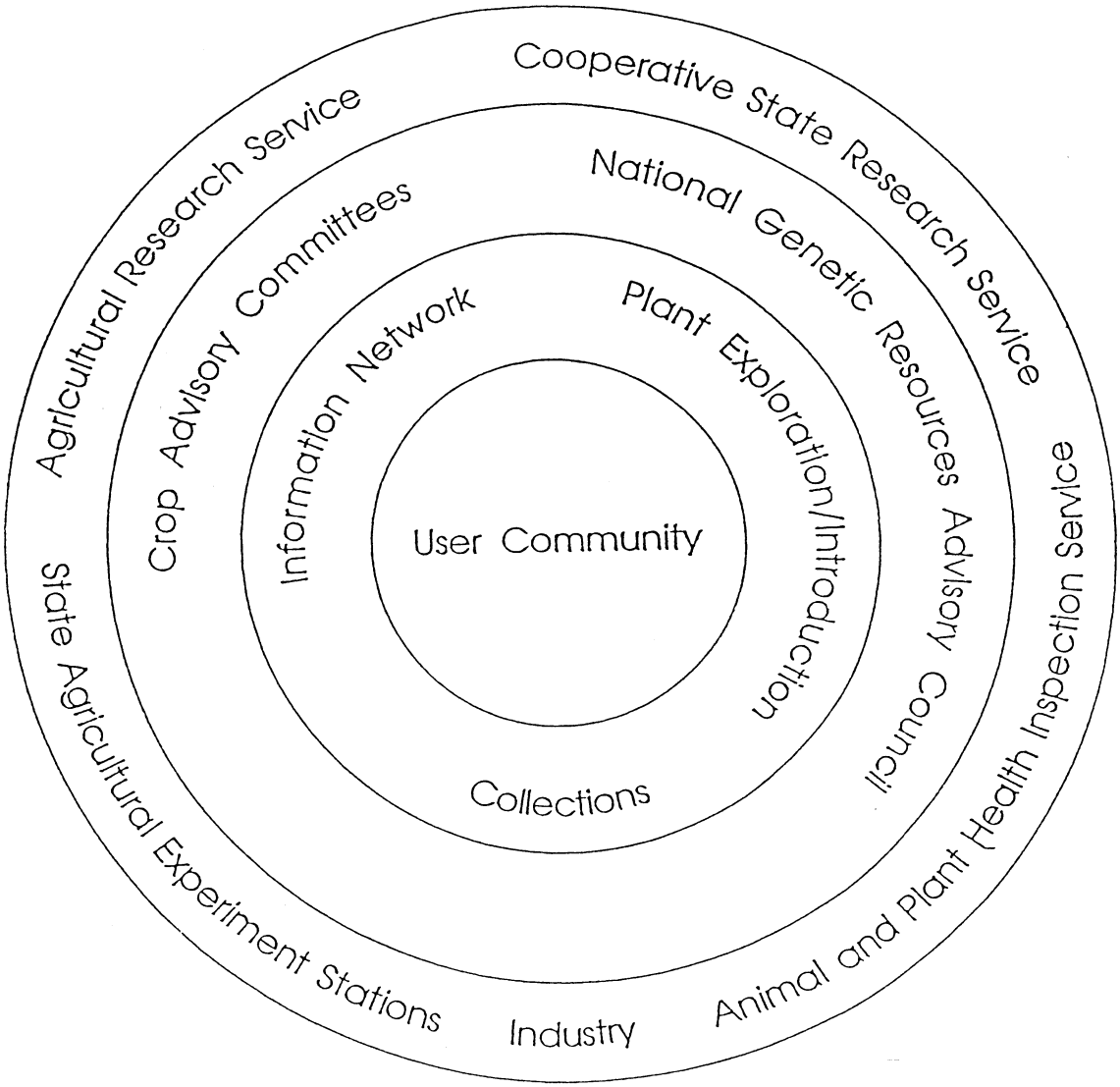


Figure 1. The U.S. National Plant Germplasm System Network-Focus and Support.

Table 1. Simple structure of the U.S. National Plant Germplasm System. August, 1993.

Working Elements of the National Plant Germplasm System:
National Germplasm Resources Laboratory
Active Collections (Seed & Clonal Repositories, Special Collections, Genetic Stock Collections)
Base Collection (National Seed Storage Laboratory)

There are several very important service functions housed within the National Germplasm Resources Laboratory (NGRL) at Beltsville (Table 2.). The underlying structure of the NPGS is a network of sites managing and servicing the germplasm program and linked together through the Germplasm Resources Information Network (GRIN). GRIN serves as the electronic connection with a database of germplasm, authoritative taxonomic listing of plant material, an order processing system and system distribution tabulator, and electronic mail for use by technicians, managers, and administrators of the system. Upgrading the capabilities of the database in the future will increase the use of the electronic medium in system management. While some changes will have significant up-front costs, the benefits over time will prove the wisdom of the changes. One example is the use of digitizd picture records of the germplasm taken at the time of collection, upon receipt, at growout, and of the germination test. Electronic storage media are becoming less expensive and pictures will be helpful to managers and users alike to supplement herbarium voucher specimens. Other services of the NGRL critical to an efficient system include: an acquisition office which deals with expeditions, introductions and germplasm management, and a quarantine center to process germplasm and assure to the country that it is free from exotic pathogens and insects. ARS and APHIS share in the responsibilities at the National Plant Germplasm Quarantine Center at Beltsville and a special satellite center for field quarantine introductions of sorghum and maize on St. Croix, Virgin Islands under the direction of the Germplasm Introduction Unit at Mayaguez, Puerto Rico.

Table 2. Components of the National Germplasm Resources Laboratory, Beltsville, Maryland. August, 1993.

Elements of the National Germplasm Resources Laboratory:
Germplasm Resources Information Network (GRIN)
Plant Exploration Office
Plant Introduction Office
Plant Quarantine Office
Crop Advisory Committee Facilitation

Of the working elements of the NPGS, the active sites form the nucleus for the interface of the user community with the system through the database. The active sites may be for seed (Table 3) or vegetaive (Table 4) collections or both , multiple or unitary crops (Table 5) or a genetic stock collection (Table 6). They interact closely with the National Genetic Resources Laboratory and the National Seed Storage Laboratory. Some of the major collections are listed (Table 7). These sites manage the collections, conduct research and provide and/or process most the data entering the database regarding their crops. Growouts are increasingly being shifted to Western states under dry but irrigated conditions to gain production efficiencies and obtain improved seed quality and quantity. Growouts in humid zones often result in seeds carrying disease organisms or of lower quality due to drought or temperature stress. This usually results in lower seed germinability making them less suitable to enter into the long-term storage genebank or be eligible for phytoasnitary certificates for shipment. The system needs no longer fit well within the political boundaries of the cooperative state system. As. activities are shifted to develop stronger efficiency in the system, the state system might shift to National Research Support Project funding to alleviate pressure on the Regional funds. Also, active sites are developing more capacity for long-term storage for their small seed size species, for their less-used accessions, and for their non-core and reserve collections to gain more efficiency.

The USDA's National Seed Storage Laboratory at Fort Collins, Colorado serves to house the U.S. base collection and conduct research related to storage methodologies and the collection's health and stability. Active collection sites arrange for growout and manage distribution of the collections.

Table 3. Accessions held at the Regional Plant Introduction Stations' active collections. August, 1993.

Location	No. Accessions
Ames, IA	40,844
Griffin, GA	67,344
Geneva, NY	12,944
Pullman, WA	52,304
Total	173,436

Table 4. Accessions held in active collections at National Clonal Germplasm Repositories. August, 1993.

Location	No. Accessions
Brownwood, TX	191
Corvallis, OR	7,779
Davis, CA	5,884
Geneva, NY	4,744
Hilo, HI	549
Miami, Fl/Mayagiiez, PR	5,341
Riverside, CA	901
Washington, DC	1,665
Total	27,054

Table 5. Some crop specific active collections of the U.S. National Plant Germlasm System. August, 1993.

Location	No. Accessions
National Small Grains Collection	118,055
Soybean Collection	14,228
Cotton Collection	6,085
Potato Collection	5,232
Flax Collection	2,659
Tobacco Collection	2,076
Total	148,335

Table 6. Some genetic stock active collections of the U.S. National Plant Germplasm System. August, 1993.

Crop	Location
Barley	Ft. Collins, CO
Brassica	Madison, WI
Clover	Lexington, KY.
Cotton	College Station, TX
Lettuce	Salinas, CA
Maize	Urbana, IL
Pea	Geneva, NY
Pepper	Las Cruces, NM
Sorghum	College Station, TX
Soybean	Ames, IA
Tomato	Davis, CA
Wheat	Columbia, MO

Table 7. Some selected agricultural crops maintained in active collections of the U.S. National Plant Germplasm System (NPGS). August, 1993.

Crop	Accessions	Location
Wheat	46,776	Aberdeen, ID
Corn	28,401	Ames, IA
Soybean	15,981	Urbana, IL
Potato	11,072	Sturgeon Bay, WI
Tomato	9,969	Geneva, NY
Peanut	8,394	Griffin, GA
Alfalfa	7,095	Pullman, WA
Cotton	6,834	College Station, TX
Grape	4,188	Davis, CA/Geneva, NY
Apple	3,952	Geneva, NY
Pear	2,004	Corvallis, OR
Citrus	779	Riverside, CA

CACs provide important guidance to the management needs of germplasm of the commodities represented (Table 8). They cover all sectors and many disciplines (Table 9). They provide useful reports to system managers and administrators (Table 10).

One of the strengths behind the present USDA system is the strong commitment of ARS to research. Research on conservation biology issues of biochemistry, genetics, and seed and plant physiology drives the system at Ft. Collins and other NPGS sites. Too little is known about: the biological basis of seed survival despite some major breakthroughs in recent years;

Table 8. Current status of NPGS Crop Advisory Committees. August, 1993.

No. of CAC's:		40
Members (excluding *Ex-Officio*):		475
ARS	109	
State	213	
Private sector	130	
Other (NGO, foreign, IARC)	23	
Ex-Officio : Curators, NPS		
No. members per CAC:	Range (6 to 18)	

Table 9. CAC composition by discipline. August, 1993.

Discipline	Number
Botany	11
Chemistry	2
Entomology	14
Food science	3
Genetics	344
Microbiology	1
Nematology	1
Pathology	41
Physiology	6
Virology	6

Table 10. Suggested CAC Annual Report Contents for Administrators and Managers of the NPGS.

Accomplishments:
New accessions
Regenerations
Distributions
Evaluations
Priority Needs:
Collection
Regeneration
Evaluation
Enhancement
Plan of work for next year
Issues requiring special attention

and cryopreservation of vegetatively-propagated germplasm; the impact of diseases harbored in the seeds; genetic shifts in storage; and, population sizes for regeneration and retention of integrity of landrace populations. Research is essential for quarantine aspects of plant introduction and distribution. Plant collecting and exchanges of germplasm between scientists have been the primary means of germplasm distribution since the commencement of formal institutional plant breeding efforts. All nations are vulnerable to importing and spreading damaging exotic plants, diseases and insects. Research requires considerable support from the performing organization and from the government planners recognizing the importance and risks of new introductions. New molecular technologies give the research community tools to attack priority diseases to determine the causative organism and its etiology and to develop the protocols to deal with it.

Management tools must receive more attention. New research is needed to develop criteria and protocols for more efficient and cost-effective ways for conservation of the biological materials. Development of rational criteria by which the "core collection" concept is implemented is critical to the commodity groups. Little is known about the practical application of the core collection concept. Recent examples in the United states

where breeders were searching for sorghum greenbug biotype I resistance, resistance to a new form of barley stem rust attacking the T gene, and wheat and barley resistance to the Russian wheat aphid suggest that the predicted 70 percent of genetic diversity contained in a hypothetical core collecion would have been inadequate to provide the breeders the resistant germplasm sources they needed. In each case, the frequency of resistant germplasm found in the gene bank was less than one percent. Strategies for constituting a core collection with a higher frequency of total diversity need to be developed. An assist from molecular markers may be necessary to supplement more conventional data and statistics. Unfortunately, it is easy to look to the molecular technologies as being the solution to all problems while forgetting that the cost of molecular analysis is high and that, for large collections , there may be a physical limits to getting a job done in a reasonable time and cost.

Large collections accumulated may create major management problems for a system. It is easy to rationalize the need for adding more and more. Some of the traditional logic: 1) we can't count on being able to receive the genetic resources in the future from some country or region due to genetic erosion, politics, and so on; 2) we can get it now, store it, and rationalize it with our present collection later; 3) we have a foolproof way to eliminate the seedborne viruses (bottlnecking the collection); and 5) the advisory committee recommends that we should get it.

Breeding developments in the future will depend on efficiencies derived from utilizing higher technologies and greater information exchange. Genome mapping is advancing rapidly and will play an important role in speeding future crop development successes. Genetic stock collections established for many crop species provide unique mutant marker gene reference points and have potential importance for deciphering genetic codes, and orienting, verifying, and effectively using the comparative species maps.

With the competition of many societal needs for funds, how can we do our jobs better with less real money in the future? The answers may lie in responses to a series of questions. What international cooperation is possible given the problems of environment, pathogens and quarantine, and politics in exchanges. What role can the International Agricultural Research Centers (IARCs) play? Is the real cost of conservation the cost of doing it, or the cost of not doing it and when will it be too late to make the decision? What is the cost of genetic materials unavailable for use? What is the cost of lost viability of samples? What is it the real cost of

diverting the limited funds to acquisition activities (quarantine growouts, greenhouses, isolation) while the present samples need more characterization to improve usefulness? A number of the questions relate to symptoms of an over ambitious collection policy and the lack of the discipline to complete the job at hand.

In 1993, a survey of CACs revealed that over half of them felt that their genetic base was too narrow and that additional germplasm is needed. At the same time their priorities were 1) to better characterize the existing collection, 2) expand growout facilities in field and greenhouse, 3) enter accessions into the database at repository sites, and - to a lesser degree - 4) clean up disease-infected collections. A relatively small number felt that a core collection was needed for their crop. From a management viewpoint, this pressure of acquiring more must be rationalized with system capacity and quality work.

Forthcoming Legal Issues on Germplasm Rights

Agriculture's capacity to provide food, clothing, shelter, fuelwood, some phytomedicinals and industrial chemicals depends on a large extent on conservation and breeding efforts. Changes in social and industrial policy must arrest the genetic erosion and the conservation effort must preserve what we have. Effective breeding strategies and procedures must be developed utilizing new technologies in the most efficient ways. Gains in productivity over the past half-century have been the result of a combination of many factors in management and improved plant types. Production increases in developing countries resulting from new management practices and breeding germplasm releases by IARCs has been unprecedented. Developed countries have also benefitted from utilizing some of these breeding materials. No matter how one looks at those gains, the composite result has come from germplasm exchanges between developing and developed countries and a commitment of countries to work together.

There is a serious concern about the access to and exchange of germplasm in the future. Recently, with the advent of biotechnology patents, intellectual property issues have complicated the exchanges and left, unresolved, a protocol for the future, The U.S. has several laws affecting property protection for plant germplasm (Table 11). Within the

U.S., the State public sector is being pressed to recover costs in the genetic resources sector just as in other sectors. Thus, the pressure for product sales to recover revenue in the State public sector emulates that of the private sector and has raised the question what is the private sector when the public organizations enter competitively into the marketplace against the private sector. This changing interest of the State universities has left the plant breeding and germplasm communities with much uncertainty and concern. In time, potential profit centers of state experiment stations might be sold off as was done with the Cambridge Plant Breeding Institute in the United Kingdom. Meanwhile, the Federal sector, under terms of the Technology Transfer Act of 1986, will likely also be under additional pressure to recover costs in some way, perhaps through increased partnerships with industry. Other countries are also struggling with the need to generate research funds not at the expense of the taxpayer.

A future protocol will also be concerned with how countries will work with each other on matters involving access to genetic resources and, in particular, those genetic resources having intellectual property protection of some form. The expectation of large profits is driving some countries to seek compensation for providing basic germplasm resources from which commercialization utilizes some critical component. The IARCs have already declared that the genetic resources provided freely to them are in trust to benefit the world's people. An emerging material transfer agreement (MTA) of the IARC's will likely have a provision that any genetic material exchanged cannot be protected in any way to prevent its use by others.

The United Nations Environment Programme's Convention on Biological Diversity (CBD) provides a legal umbrella to request compensation for providing genetic resources. Countries must recognize that instituting charges for genetic resourecs may be more costly long run. It is to the benefit of the agricultural economies of all nations to continue to exchange germplasm resources freely. Relationships between the legal status of the CBD and FAO International Undertaking on Plant Genetic Resources in areas affecting exchange are compared by the bilateral-multilateral arrangements, the charge-free for exchange, and the time of implementation (Table 12). Due to the interdependence of countries for different commodities, all nations will continue to be best served well through free and open exchange. There are countries failing to exchange freely at this time and others will likely do so in the future. The marketplace will bring that into balance at some point in time. Payment for

access is what the CBD advocates. What happens to the poorer countries, the less able countries, the less prepared countries? The "Lost Decade", when the FAO issues clouded many activities, must not be repeated for the conservation aspects of plant genetic resources. Fortunately, the IARCs continued about their business: production agriculture.

There is an opprtunity for the IARCs and their donor supporters to build upon Center strengths to make some essential changes in genetic resources conservation and crop protection. While individual IARCs have recognized the eco-regional conservation need, there is not adequate funding to expand this to a broader genetic resources base and conservation of minor and local crops, and their relatives. The donor organizations have the responsibility to urge development of regional implementation plans to collect, preserve, and utilize--in concert with the nations of the region--genetic resources of importance to the agro-ecosystems of the world, to conduct research and provide training for the participants from those nations, and to press for a more sustainable agriculture in all countries. Utilization of modern biotechnologies as employed in the IARCs will enable the technologies to be instituted in developing country programs. As national programs become competitive globally, there will be increasing interest in establishing an adequate and effective intellectual property system to enable the countries to protect, exchange, and benefit from their works. In the meantime, there will likely be continued difficulties to exchange and protect material.

There will be a renewed interest on the part of the Conference of the Parties to the Convention on Biological Diversity to show that it is instituting action plans on the scientific and legal fronts. Scientists the world over must do their part to collaborate and keep the germplasm flowing as freely as possible while recognizing that they are exchanging germplasm, technologies, and information on mutually agreed terms. Any national legislation which interrupts this flow through increased bureaucracy or restrictive regulations will do that country and the world a great disservice. The Conference of Parties should acquire agricultural expertise through sub-committee to assure that the world's people have access to a continued bountiful food and agricultural production through better conservation of genetic resources and development of improved crop cultivars and hybrids.

Table 11. Intemational and U.S. Legal Protection for Plant Genetic Resources.

	UPOV Convention	Patent Law	Proposed Federal Research Exemption
Legal Act:	Plant Variety Protection Act	Utility Patent Act; Plant Patent Act	Utility Patent Act Proposed Federal Reqt.
Int' Nat'l:	Multilateral	Bilateral	Bilateral/Multilateral
Access Terms:	Open & no cost- No infringement for Research/Breeding	Terms of Material Transfer Agreement (MTA)	Open & no cost- Universal MTA Terms
Commercailization	Essentiall derived requirement	Terms of MTA	Universal MTA Terms

Table 12. Options of Access to Genetic Resources under International Conventions.

	FAO Undertaking	UNEP Convention	UNEP Convention
Treaty:	Non-binding	Binding	Binding
Arrangement:	Multilateral	Bilateral	Bilateral
Access option:	Open and no cost	Open and no cost	Payment for access
Terms:	None; No direct commercialization	Royalties upon commercialization	Royalties upon commercialization

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THE CONSERVATION OF BIOLOGICAL DIVERSITY

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Natural resources are vital to sustaining the world's human populations. Biological resources are key to conserving water, soil, and air quality, but are often overlooked in soil and water conservation projects. The millennia of natural selection and adapation to local environments is expressed in great genetic diversity among and between species. Development, in the economic and social sense, in recent decades has perceptibly increased the homogenization of the worrld's human needs and desires. This is also occurring with heavily used biological resources. such as plants used as crops and animals grown for food. Since future development of crops and livestock depends fundamentally on gene or allelic diversity, the need for conservation of biological resources is obvious. Furthermore, developments in gene transfer technology have supported the dogma of "any gene to any species". Thus, all biological resources become candidates as gene resources to meet human needs. Therefore, conservation of biological diversity for its eventual use by humans is not simply a responsibility of the practitioners of agriculture and forestry, the traditional users of genetic resources, but is extended to the global human population. The movement to conserve biological diversity has gained momentum, but unfortunately, there is polarization between those who are concerned with the conservation of nature in general and those whose primary activities are to exploit biological resources or their natural habitats for urban and industrial uses, for agriculture, for water development projects, or surface mineral mining. A global biological conservation plan must expect dedication to conservation practices at international government levels all the way to individuals in villages and communities. Incentives are required beyond "for the good of nature". Endowments or trusts provide the best insurance for perpetual care of biological resources beyond the life of a governmental regime or particularly influential individuals. There ar inevitable compromises to be made and some biological resources will be lost. The goal of avoidance of gene losses by strongly integrated *in situ* and *ex situ* conservation seems to operationally and socially acceptable. The framework for the facilitation of the integrated approach is the main theme of this presentation.

Plant Germplasm Conservation: Perspectives for the 2000s. 17-28 (1994). Taiwan Agr. Res. Institute

OPERATIONS AND MANAGEMENT OF THE NATIONAL PLANT GERMPLASM SYSTEM IN JAPAN

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After a remarkable success was performed in varietal improvement of major crops since 1930's, our country was faced irreparable losses of plant materials especially in rice, soybean, wheat and barley. The value of landraces was understood and had as to be preserved in the future materials. Researchers of the various breeding groups then made efforts for safe conservation of their materials.

Rapid modernization of agricultural practices and development of transportation made further attacks on the genetic erosion even in vegetables and minor crop species since 1960's. The time was then ripe for scientific reform on plant breeding in universities, public organizations as well as in the institutes under the Ministry of Agriculture, Forestry and Fisheries (MAFF). Establishment of a new system for the research required a new organization and budget and network systems which could be coordinated with introduction, collection, evaluation, preservation, distribution and utilization of plant genetic resources.

1. The MAFF Genebank System

The Genetic Resources Center of MAFF was established in the National Institute of Agrobiological Resources (NIAR, Tsukuba) in December 1986, by reorganizing the former Department of Genetic Resources. The former MAFF Genebank Project, which was started in 1983, has been acclerated to solve urgent problems in overall management of genetic resources.

A 10-year plan of the MAFF Genebank Project includes not only plants but also microorganisms, animals, forest trees, and fisheries organisms. NIAR is the central organization for plants, microorganisms and animals. These three areas are coordinated by the Genetic Resources Coordinator in the Center. NIAR's Genetic Resources Center is in this way playing a uniting role in getting close cooperation with co-working research organizations.

2. Network of Plant Genctic Resources in MAFF

The MAFF Genebank consists of a National Center (NIAR) and 15 Sub-banks located throughout Japan. Sub-banks are in 15 institutions, i.e., 13 National Research institutes. including NIAR, the National Center for Seed & Seedlings, and the National Livestock Breeding Center, as shown in Table 1. They are assigned specific crops, and share research activities on collection, evaluation and preservation of vegetatively propagated plants, multiplication of plant materials, and development/utilization of new breeding materials, as shown in Table 2. Prefectural institutes of 43 research units are connected to the network of the Project by special assignment with MAFF, covering exploration and collection abroad, as well as evaluation and multiplication of specific crops.

Crop species or plant groups in the Project are practically divided into 12 groups i.e., rice, wheat/barley, tuber crops, legumes, miscellaneous small grains/industrial crops, forage crops, fruit trees, vegetables, ornamental plants, tea, mulberry and tropical plants. Thus, 12 curators have been appointed for each plant group, as shown in Table 3. They belong to various institutes as a representative of each plant group, making annual plans, long term schemes for evaluation, documentation and collection of plant genetic resources as well as proposing appropriate budgeting, etc. The relations between the Center bank and the curators, in addition to the verical connection of Center and Sub-banks system, make it possible to carry out the MAFF Genebank Project effectively.

3. Introduction and Collection

Introduction and collection of genetic resources was gradually accelerated, first by universities, and secondly by individual institutes such as Tropical Agricultural Research Center (TARC), MAFF. However, no systematic introduction nor collection was organized until the MAFF Genebank Project was started. The MAFF Project supports several missions every year to collect in various regions of Japan, mainly for minor and vegetative crop species. International expeditions for germplasm collection are carried out by cooperation with local research organizations. Since 1983, we have sent 4-5 missions abroad every year, and made joint teams with local institutes for field collection and exploration by direct support of the Project

(Table 4). Additionally, international missions were actively organized by different budget sources from IBPGR, TARC, Japan International Cooperation Agency (JICA) and others (Tables 5).

4. Preservation

In 1968, we constructed the first modern genebank for multi-crop species at Hiratsuka. This walk-in type bank had the first system for duplicate preservation of seeds, i.e., long-term preservation for permanent and original stocks and medium-term for distribution to users. After ten years, a new bank with a robotic system was established at Tsukuba in 1978. The new bank had also two types of rooms with different conditions to preserve seed for long-term (-10℃, 30%RH) and for medium-term (-1℃, 30%RH) for distribution. After a few years we soon faced a shortage of storage capacity. NIAR then constructed the third-generation genebank with a complete robot system in 1988. The earlier bank was rebuilt after completion of the third one in 1991 to facilities for extended long-term storage and original seed stocks.

Vegetatively propagated plants need a enebank in the field or a cellbank. The former are still important to maintain fruit trees, tea, mulberry, and other vegetable crop species. The Center Bank and many Sub-banks located at suitable places are assigned to preserve their specific crops.

Cryopreservation techniques make it possible to preserve effectively plant cells, tissues, orthodox seeds for base collection and some recalcitrant seeds. The combination of a liquid nitrogen tank and a programmable freezer is the latest technical improvement, used to bulid a system for long-term preservation in ultra-low temperatures (-196～-160℃). The technique for the cryopreservation is adopted for not only microorganisms nor animal cells, but for important base collections of plant seeds. The capacity of them is still under developing.

All kinds of accessions preserved in the Project are classified into three types: base, active and working collections. Base collections are the accessions registered for long-term preservation as originals, while active collections are for distribution to users, as shown in Table 6. The working collections are still maintained at the laboratory level, in addition to those plants registered in the base collection.

5. Evaluation and Data Management

Strict characterization and evaluation of genetic resources is required for utilizing them as breeding materials, experimental lines and various other unexpected uses. Evaluation data, if extensive enough and easily available for users must be helpful for parctical use. Nevertheless, evaluation duties are not always welcomed by researchers, because they are hard and dull work.

The MAFF Genebank Project designates three levels of evaluation for each crop species, i.e., primary, secondary and specific characters, depending on the extent of importance in utilization and complication in measuring. The descriptors for the primary evaluation are limited in number to ca. ten characters as essential items. The secondary characters include resistance genes for pests and diseases, detailed agronomic traits, such as some quantitative characters and stress tolerance. The specific characters are, e.g., amylose content of cereal endosperm, enzyme activities/electrophoretic zymogram patterns, grain quality, productivity or yield of production, and etc. A record of accessions primarily evaluated in recent years is shown in Table 7.

To develop data management systems is indispensable for handling germplasm materials collected worldwide, for constructing inventory database and for utilizing evaluation data. The MAFF Project has established management systems for passport data processing, conservation/distribution of plant germplasm materials and evaluation/characterization.

6. Distribution, Exchange and Utilization

The aim of collecting, evaluating and preserving germplasm is to utilize in plant breeding and the other practical use. The process of modern plant breeding needs several steps for adding new traits, as well as exotic germplasm with detailed data. The current situation is not ideal for the breeders, but our research on plant genetic resources is directed for that goal. As records of accessions distributed from the MAFF Project show (Table 8). MAFF germplasm materials were used by not only national institutes, but also by universities, prefectural institutes, private sectors and organizations abroad.

Utilization of plant genetic resources in breeding has reached a new phase. Artificial gene transfer and recombinant DNA methods in biotechnology need new genes from well preserved materials and

accumulated research results in each crop species. The rapid development of the " Rice Genome Project " at NIAR is a good example.

7. International Exchange and Cooperation

Thinking of the size of our planet, international cooperation on plant genetic resources becomes important naturally. Networking for practical solutions is guided by IBPGR (IPGRI) and international institutes for agriculture. NIAR and cooperating institutes have been appointed as registered genebanks for the international base collection of many plant species, Japanese specialists have been participating in the IBPGR collecting missions every year. JICA, the Japan International Cooperation Agency, has also contributed much in this field by assisting establishment of genebanks and the storage facilities in Bangladesh, Thailand, Sri Lanka, Chile, Myanmar, Pakistan, etc. International training course on plant genetic resources is also one of the activities sponsored by JICA. We received 113 scientists from 24 countries until the 1 lth class in 1992.

8. The Second Phase of the Project and the Prospects

Technology of modern agriculture has reduced extent of genetic diversity and accelerated the genetic erosion in various crop species. Because of the serious environmental disruption worldwide, the ecological situation for even the wild species is going from bad to worse. New approaches will become important both for maintaining the biodiversity of plant resources and for preventing the extinction of important plant species.

The second phase of the MAFF Genebank Project was started at the beiginning of April 1993 as another 8-year plan in all areas of the genetic resources, i.e., 1) plants, 2) microorganisms, 3) animals, 4) forest trees and 5) fisheries organisms. It aims at accumulation of data on detailed characteristics, at further development of database management, at international cooperation of research exchange. Moreover, corresponding to a rapid development of the " Rice Genome Program " at NIAR, the DNA-bank Program has been initiated in 1993 for the sixth category of the MAFF Genebank Project. They are national programs, however beyond a nation if we thought the present status on the environment surrounding us. Ultimately, more attentiion must be directed to ecological aspects, so as to maintain our planet for all living creatures.

Table 1. Plant Genetic Resources System in MAFF

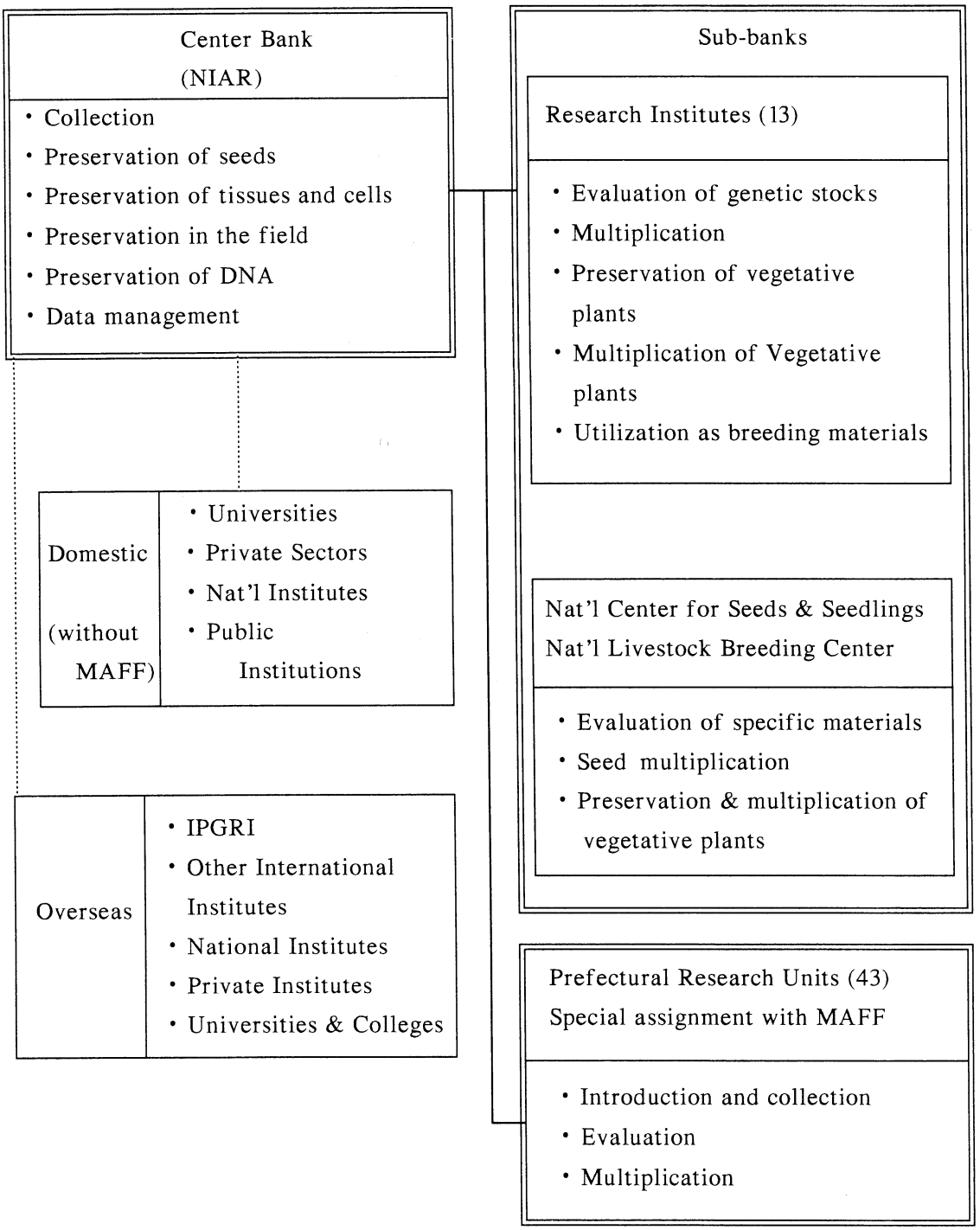


Table 2. Network of MAFF Genebanks

Center Bank = National Institute of Agrobiological Resources (NIAR. Tsukuba)

Sub-banks

Prefectural Research Units with special assignment = 43 Units

MAFF Organizations (15) Name of Sub-Bank (abbr., location)	Crop species	No. of Units Labs
National Agriculture Research Center (NARC, Tsukuba)	Rice, Wheat, Soybean. Sweet potato. Millet, etc.	11
National Institute of Agrobiological Resources (NIAR, Tsukuba)	General and Rice etc.	11
National Grassland Research Institute (NGRI, Nishinasuno)	Forage plants, Maize, etc.	5
Fruit Tree Research Station (FIRS, Tsukuba and branches)	Fruit trees	10
National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT, Anou and branches)	Vegetables, Tea, Ornamental plants	20
Hokkaido Agricultural Experiment Station (HNAES, Sapporo & branches)	Rice, Fruit trees, Forage crops, Root crops, etc.	14
Tohoku Agricultural Experiment Station (TNAES, Morioka & branches)	Rice, Soybean, Oil seed, Mulberry, etc	8
Hokuriku Agricultural Experiment Station (HNAES, Joetsu & branches)	Rice, Forage crops, etc.	5
Chugoku Agricultural Experiment Station (CNAES, Fukuyama)	Rice, Wheat, Sorghum, etc.	5
Shikoku Agricultural Experiment Station (SNAES, Zentsuji)	Rye, Wheat	2
Kyushu Agricultural Experiment Station (KNAES, Nishigoshi & branches)	Rice, Wheat, Barley, Sugarcane, Root crops	13
National Institute of Sericultural and Entomological Sciences (NISES, Tsukuba)	Mulberry	3
Tropical Agricultural Research Center (TARC, Tsukuba & Ishigaki)	Tropical crops, Rice, etc.	4
National Center for Seed & Seedlings	Oats, Fruit trees, Potato, Tea, etc.	Farms 13
National Livestock Breeding Center	Forage crops, Maize, etc.	Ranches 3
		Total 170 Labs & units

Table 3. Curators of Plant Genetic Resources in MAFF Genebank

- Introduction, Collection
- Evaluation, Multiplication
- Preservation
- Data management

NIAR Labs in Center Bank

Crop group	Crops targetted	Curator (Chief of lab shown below)
01 : Rice	Rice and related species	Rice Breeding Technology Lab., NARC
02 : Wheat & Barley	Wheat, Barley, related species	Wheat and Barley Technology Lab., NARC
03 : Legumes	Soybean, Adzuki, other beans & nuts	Legume Breeding Lab., NARC
04 : Tuber crops	Sweet potato, Potato, others	Sweet Potato Breeding Lab., NARC
05 : Millet & Industrial crops	Miscellaneous cereals Oil crops, etc.	New Crop Breeding Lab., NARC
06 : Forage crops	Forage crops	Breeding Material Lab, NGRI
07 : Fruit trees	Fruit trees	The 4th Lab of Breeding, FTRS
08 : Vegetables	Vegetables	The 4th Lab of Breeding, NIVOT
09 : Ornamental plants	Flower plants and other ornamentals	The 1st Cut Flower Lab of NIVOT
10 : Tea	Tea plants	Breeding Lab, Tea Cultivation Div., NIVOT
11 : Mulberry	Mulberry plants	Mulberry Breeding Technology Lab., NISES
12 : Tropical plants	Tropical and Sub-tropical plants	Crop Introduction Lab, TARC

Table 4. International collecting missions organized by MAFF Genebank Project*

Year	Areas	Target plants
1980	1:European countries	*Beta,* Potato
1981	1:Ivorv Coast. India	Rice, Sorghum
	2:Papua New Guinea, Solomon Is.,	Taro, Yam, etc.
Fiji		Ryegrasses, Alfalfa, etc.
1982	1:Italy, France	*Amorphophallus conjac*
1983	1:Thailand, Philippines	Soybean
	2:Indonesia	Wheat, Barley
	3:Turkey, Egypt	Tomato, Pimento
	4:Mexico, Costa Rica, Peru, Bolivia	Rice
	5:Bangladesh	Tea
1984	1:India, Thailand, Indonesia	Grapes, etc.
	2:North America	Rush
	3:New Zealand	*Vigna* spp.
	4:Korea (s.)	*Prunus. Pyrus, Diospyros*
1985	1:Taiwan	Wheat, Barley
	2:Italy, Hungary	Sweet potato, Sugarcane
	3:Papua New Ginea	Peanut, Maize
	4:Argentine	Soybean
	5:Thailand	Forage plants
1986	1:Morocco, Portugal, Spain	Rice
	2:Thailand	Root crops, Vegetables
	3:Peru, Ecuador	Small millets
	4:Tanzania	Vegetable crops
	5:Thailand	Vegetables
1987	1:Nigeria	*Vigna* spp.
	2:Nepal	Wheat, Barley
	3:Morocco, Syria	Citrus, Barley
	4:Italy, Greece, Israel	Citrus, Loquat, etc.
1988	1:Nepal	Rhododendron, Lilium
	2:India	Sugarcane
	3:Argentina	Alfalfa
	4:Indonesia	Upland rice
1989	1:Algeria	Wheat, Barley
	2:Malaysia, Thailand	Sweet potato
	3:France, Italy	Grapes
	4:Greece	Tulip, Allium
1990	1:Morocco, Spain	Wild *Beta*
	2:Finland, Sweden	Forage plants
	3:Thailand	Rice
	4:Malaysia	Food legumes
1991	1:USSR	Forage plants
	2:Poland	Vegetables
	3:Sri Lanka, Thailand	Millets
	4:Uruguay, Chile	Root crops
1992	1:Brazil	Pineapple
	2:Mexico	Mulberry
	3:India	*Seasmum*
	4:Madagascar	Rice

*: MAFF Genebank Project, i.e. Genetic Resources Project, MAFF is coordinated by NIAR under AFFRC, MAFF. The Missions funded by the project are internationally conducted including a Japanese team leader, Japanese members and members from host countries.

Table 5. Collecting missions with other funds (selected. led by Japanese Team Leaders)

Time	Fund and status	Area	Crop or Genus
1980	TARC, Bilateral	Mexico, Costa Rica, Peru, Brazil, Ecuador, Colombia, Panama	Tuber crops
1982	TARC, Bilateral	Papua New Guinea, Fiji, Solomon	Tuber crops
1983	IBPGR, International	Thailand	Citrus spp.
1983	TARC, Bilateral	Malaysia, Thailand	Tropical vegetables
1983	IBPGR, International	Vietnam, Laos	Food legumes
1984	IBPGR, International	Thailand, Malaysia	Citrus spp.
1984	IBPGR*, International	Nepal	Cereals, Fruits, Vegetables
1985	TARC, Bilateral	Colombia	Forage crops
1985	IBPGR*, International	Nepal	Cereals, Fruits, Vegetables
1985	TARC, Bilateral	Philippines, Malaysia	Tropical vegetables
1986	IBPGR*, International	Thailand, Indonesia	Citrus spp.
1986	IBPGR*, International	Papua New Guinea	Tuber crops
1987	TARC, Bilateral	Syria, Turkey	Food legumes
1987	IBPGR*, International	Papua New Guinea	Tuber crops
1988	IBPGR, International	Indonesia	Citrus spp.
1989	TARC, Bilateral	Thailand	Tropical fruits
1989	IBPGR*, International	Pakistan	Cereals, Legumes, Millets
1990	TARC, Bilateral	Cote d'Ivoire, Tanzania, Kenya	Forage crops
1990	TARC, Bilateral	Indonesia, Thailand	Tropical fruits
1991	IBPGR*, International	Pakistan	Cereals, Legumes, Millets
1992	TARC, Bilateral	Indonesia	Fruit trees
1992	IBPGR*, International	Russia & neighboring republics	Fruits, Forage
1993	TARC, Bilateral	Indonesia	Sugarcane
1993	IBPGR*, International	Russia & neighboring republics	Wheat, barley,
1993	IBPGR*, International	-do.-	Legumes, Vegetables, etc.

TARC : Funded by Tropical Agricultural Research Center, AFFRC, MAFF.

IBPGR : Funded by IBPGR as International mission.

IBPGR* : Organized by IBPGR with special funds of Japanese government.

Table 6. Present Status of Preservation of Plant Genetic Resources under the MAFF Genebank (1992)

Crop group	No. of accessions preserved			Collection preserved vegetatively
	Total collection	Base collection	Active coll.	
Rice	26, 570	22, 695	16, 744	120
Wheat and barley	54, 509	50, 035	23, 242	274
Food legumes	14, 688	12, 395	8, 605	0
Root and tuber crops	5, 667	4, 780	3, 548	5, 023
Millet oill & industrial crops	9, 728	7, 553	5, 583	2, 092
Forage plants	42, 444	17, 441	11, 052	8, 853
Fruit tress	7, 851	5, 553	3, 832	7, 850
Vegetables	20, 540	11, 648	5, 816	1, 366
Flower and others	2, 864	842	239	2, 857
Tea plants	5, 353	4, 354	496	5, 353
Mulberry	1, 855	1, 406	209	1, 855
Tropical crops	791	127	126	121
Total	192, 860	140, 829	79, 492	35, 764

Table 7. A Record of Accessions Evaluated Primarily under the MAFF Genebank Project in Recent Years

Crop group	1986	1987	1988	1988	1990	1991	1992
Rice	1, 194	1, 307	1, 021	902	914	1, 093	1, 420
Wheat and barley	3, 086	3, 296	5, 387	5, 132	3, 010	3, 708	3, 576
Food legumes	328	433	512	484	550	546	802
Root and tuber crops	723	4, 800	4, 336	5, 311	4, 170	4, 710	4, 899
Millet, oil & other crops	424	974	984	749	1, 346	1, 614	2, 764
Forage plants	869	1, 169	1, 947	1, 410	3, 157	1, 706	2, 595
Fruit tress	572	854	1, 124	681	657	760	613
Vegetables	833	777	968	865	897	2, 233	1, 141
Flower and others	475	330	216	303	853	488	420
Tea plants	280	294	474	474	1, 588	1, 934	3, 328
Mulberry	145	260	375	431	285	276	243
Tropical crops	50	121	23	89	44	44	29
Total	8, 979	14, 615	17, 367	16, 831	17, 471	19, 112	21, 830

Table 8. Records of Accessions Distributed in Recent Years

Year	Acc. distributed to national organizations			Abroad	Total
	Institute	University	Private sector		
1985	198	0	57	266	521
1986	4, 515	1, 344	295	811	6, 965
1987	9, 811	356	432	1, 548	12, 138
1988	3, 472	229	735	1, 309	5, 745
1989	14, 082	480	397	254	15, 213
1990	5, 651	896	482	373	7, 402
1991	5, 394	850	380	691	7, 315
Total	43, 123	4, 155	2, 769	5, 252	55, 299

Plant Germplasm Conservation: Perspectives for the 2000s. 29-47 (1994). Taiwan Agr. Res. Institute

THE MANAGEMENT OF VEGETABLE GERMPLASM COLLECTIONS[a]

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Introduction

Vegetalbes makeup a complex crop group. Vegetables in the broad sense belong to several families of plants, including legumes and tubers, Easily a list of vegetable species can run into the hundreds. This is not surprising considering that 1,500 wild species may have been involved in the evolution of vegetable species (Grubben, 1977). Many of these species are annuals whose leaves, stems, immature flowers, fruits, seeds, roots or tubers are consumed alone or in combination with other foodstuff usually as supplementary source of vitamins, minerals, fibers, additional proteins and calories.

The recognition of the importance of vegetables in proper nutrition and their potential to raise incomes suggest that vegetable production will increase especially in developing countries where consumption is estimated to be only half of that in developed countries (Grubben, 1977).

Vegetables are in various stages of domestication. The continuum starts from wild populations harvested when needed and includes semi-wild partially cultivated as well as varieties of traditional agriculture and extends to highly specialized horticultural varieties.

As in other crop species, the conservation of vegetable genetic resources for preservation and use in crop improvement is essential 1) if we are to enhance agricultural productivity especially in rural and urban areas of developing countries and 2) if we are to answer future needs.

The importance of vegetable genetic resources in present vegetable genetic improvement programs is reflected in the amount of materials that

the Asian Vegetable Research and Development Center (AVRDC) has distributed (Tables 1 and 2). In 1992 alone, 85 countries and territories received AVRDC vegetable germplasm. A total of 19,283 samples were distributed outside of the Center. Within the Center, 4,247 samples were utilized. The annual increase over the last two years was 4,000 samples (AVRDC, 1992, 1993). Samples distributed included not only the principal crops but also non-principal crops like minor legumes, amaranths, etc. However, an assembly of vegetable germplasm is meant to answer not only our present but also our future needs.

This paper will present strategies in the management of vegetable germplasm collections for use in preservation and distribution. It is based mainly on the experiences of AVRDC.

Table 1. External seed distribution of germplasm and breeding lines in 1992.

Country	No. of samples							
	Soy-bean	Mung-bean	To-mato	Chinese cabbage	Pepper	Sweet potato	Others	Total
Thailand	2,176	2,298	2,025	371	419	-	266	7,555
India	15	115	386	1	460	52	1	1,030
Philippines	25	352	70	18	485	20	1	971
Taiwan	16	7	-	3	56	644	64	790
Peru	-	-	-	2	-	697	-	699
USA	40	67	135	2	182	56	167	649
Korea (s.)	199	11	352	-	57	-	5	624
Tanzania	-	40	331	28	-	100	-	499
Sri Lanka	38	14	136	-	277	25	-	490
Fiji Islands	20	40	369	3	-	-	-	459
Others[a]	811	1,259	1,677	321	1,052	345	52	5,517
Total (85)	3,340	4,203	5,508	749	2,988	1,939	556	19,283

[a] Includes 75 countries and territories.

Table 2. Internal seed distribution at AVRDC in 1992.

Department	Soy-bean	Mung-bean	To-mato	Chinese cabbage	Pepper	Egg-plant	Others	Total
Analytical Lab	1	1	-	-	-	-	-	1
Entomology	17	2,038	1	-	250	101	-	2,407
Pathology	258	107	421	191	439	47	11	1,474
Physiology	-	2	11	-	-	-	-	13
Plant Breeding	22	6	14	30	61	-	-	133
Training	-	-	24	25	14	-	12	75
Crop Management	-	-	20	1	-	45	2	68
Country Program	-	-	-	-	-	45	16	61
GRSU	-	-	-	-	15	-	-	15
Total	298	2,153	491	247	779	238	41	4,247

AVRDC and It's Mission

The management of a germplasm collection is made much easier if the aims and objectives of the unit responsible for it are clearly stated. A genebank which is part of an institution clearly must be committed to the mission of that institution.

AVRDC's mission for the 1990's is "to enhance the nutritional well-being and raise incomes of poor people in the rural and urban areas of developing countries through improved varieties and methods of vegetable production, marketing and distribution, which take iinto account the need to preserve the quality of the environment" (AVRDC, 1992). To attain this goal, AVRDC has identified three broad categories of activities, one of which is the "conservation and characterization of vegetable genetic resources and their use to develop improved varieties." The germplasm collection of the Center is therefore geared towards preservation and utilization.

The Scope of the Collections

Table 3 shows the status of the AVRDC vegetable germplasm collec-

tion. The AVRDC collections represent a unique and invaluable regional and world resources of vegetable. We have assembled one of the largest vegetable genebanks and has accepted responsibility for the world base collection of mungbean and the global duplicate collection of pepper. The collection was assembled primarily because of the need for germplasm in crop improvement programs of both the Center and that of NARS (National Agricultural Research Systems).

Table 3. The AVRDC germplasm collections as of 30 September 1993.

Crop	No. of accessions
Priority crops:	
Sorybean (regional)	13, 603
Tomato	6, 629
Pepper	6, 363
Mungbean (regional)	5, 737
Brassica	1, 421
Eggplant	564
Allium	327
Subtotal	34, 644
Other crops:	
Yardlong bean and cowpea	1, 600
Black gram	452
Phaseolus bean (including lima)	450
Luffa	229
Pea	207
Cucumis	206
Abelmoschus	187
Lablab bean	161
Cucurbita	148
Benincasa	126
Others	919
Subtotal	4, 685
TOTAL	39, 329

A utilization-oriented collection, whether to serve present needs or that of the future, should include as wide a range of diversity as possible.

Vegetable breeders and other researchers require genetically diverse materials to be able to continuously develop improved types and varieties that can face the challenge of an environment that is both evolving and becoming limiting. A genetically diverse genepool is the raw material from which the plant breeder will mold new varieties. Therefore, the availability of useful genes in the collection determine the success of the plant breeder in fashioning out a variety with specific characteristics. The more diverse a genepool is, the higher is the probability that it would contain the desirable genes.

Although AVRDC's collection of vegetable germplasm is considered one of the most extensive in the world, there are still obvious gaps. Even for its principal crops, the collection is deficient in members of the secondary and tertiary genepools as well as other members of the primary genepool. Representation of non-principal, but nevertheless regionally important, vegetable crops range from inadequate to practically non-existent. Therefore, to be able to accomplish its objectives fully, AVRDC is stepping up its germplasm acquisition and collecting efforts. There is an added urgency to undertake these efforts in developing countries such as those in Southeast Asia because of the increasing threat of genetic erosion (Sastrapradja and Kartawinata, 1975). The pressures of development, increasing population and vigorous marketing of modern cultivars by seed companies are resulting in the rapid disappearance of old local varieties and the old species which may carry valuable genetic characteristics.

In recognition of the need for collecting in Southeast Asia, AVRDC stepped up its exploration activities in Malaysia, Philippines, Thailand and Indonesia as part of a JICA-supported project "Conservation, Evaluation and Utilization of Vegetable Genetic Resources - A Collaborative Network Project for Southeast Asia." To implement the project, AVRDC sought the collaboration of national institutions in the participating countries. A series of consultation was held with the national scientists to map out the strategy that will be followed in the collecting expeditions. Where available (e.g. Philippines and Indonesia), a survey of past effort and current status of germplasm resources was done. Priorities in species and geographic area were set and the route mapped out. In many of the expeditions, the strategy followed was to visit the fresh vegetable market in the morning to survey what vegetables were found in the area. To make sure that samples of diverse material found in the market were represented in the collection, a few fruits of eggplants, pepper, gourd, etc., were bought. Vendors were interviewed as to sources of materials. Afterwards, the local guide

accompanied the collecting team to vegetable-growing areas or to specific home gardens and farmlands. Most of the collected materials came from backyards or home gardens followed by collections from farmlands. Many could be classified as primitive cultivars or landraces and farmer's selection. Some wild species were also collected. As a result of the collecting expeditions, a total of 3,880 samples were collected (Table 4). Collected materials were shared: half was left in the country where they were collected and half was brought to GRSU. This project is the first step towards AVRDC's longterm objective of assembling a comprehensive germplasm collection of its principal crops, as well as the other major vegetable crops including indigenous species. While initially efforts have been concentrated on the principal crops, the Center recognizes that an international effort on vegetable should try to cover other vegetable crops species as well, taking into consideration the priorities of national programs especially in developing countries.

Table 4. Vegetable germplasm collected in Southeast Asia in 1990-92.

Country	No. of species[a]	Total no. of samples			Total
		1990	1991	1992	
Malaysia	28	448	-	-	448
Philippines	45	-	994	491	1485
Thailand	59	-	-	1974	1947
Total		448	994	2483	3880

[a] Includes only those that have been properly identified.

However, for the collection to be meaningful and useful, it has to be grown, multiplied, carefully described, evaluated, stored, and documented.

Seed Increase

Quantity of seeds to produce. New introductions frequently arrive with insufficient quantity of seeds to be directly stored for preservation. This is especially true of vegetable species collected from home gardens

and wild populations where only a few mature fruits can be collected. At least one cycle of seed multiplication is needed to produce sufficient and viable seeds for preservation and distribution.

It is best to produce large quantities of seed sufficient for preservation and distribution in as few cycles as possible. Frequent regeneration of seeds is not only costly but may also result to questionable genetic fidelity possibly due to mechanical errors, genetic drift when the sample size is small as well as genetic shifts caused by loss of unadapted genotypes (Breese, 1989).

For genetically uniform material, the acceptable size is 1,000 viable seeds within the accession and the preferred is 1,500 to 2,000 viable seeds (IBPGR, 1993). The recommended amount for heterogeneous material is 12,000 with 4,000 as minimum. At AVRDC, the practice is to produce at least 20,000 seeds per accession. This quantity is divided into two groups: 4,000 or 12,000 for the base collection under longterm storage condition of homogeneous and heterogeneous accessions, respectively, and the remainder for the active collection under medium-term preservation. To produce the target amount of seeds, 30 plants of each accession need to be established if the accession is uniform. For a heterogeneous population the number of plants used in seed increase should be sufficient to preserve its genetic composition. In cases where a sample showing distinct morphologic variants, e.g., red versus yellow mature fruits, is separated into two accessions, a second planting may be necessary to meet the required amount of seeds.

Wild species pose a problem in seed production. Supplementary hand pollination or use of insect vectors may be necessary to ensure that the desired amount of seeds is produced.

As in the collecting effort, AVRDC has taken the strategy of involving national programs in the multiplication of germplasm in its collection. Newly collected materials are multiplied in the country where they were collected. These circumvents the problems of adaptation, quarantine and cultural management of indigenous crop species. It also allows the multiplication of large numbers of accessions in a short time. Furthermore, there is the added advantage of local staff being able to immediately evaluate the potential of the materials. The Center has also sought the cooperation of countries with strong genetic resources programs in the multiplication of some of its collection: pepper multiplication in collaboration with the National Plant Genetic Resources Laboratory of the Philippines and soybean multiplication with Kasetsart University in Thailand. Recently,

AVRDC and the National Plant Genetic Resources Center of the Republic of China embarked on a joint germplasm multiplication project. Such cooperative effort speeds up the multiplication of new introductions. Seeds produced are shared between AVRDC and the collaborating institution.

Preserving genetic structure. The seed increase operations consider the need to preserve the genetic structure of the original population. Since the AVRDC collections include both self-pollinated and cross-pollinated species, seed increase procedures vary. In any case, to preserve the genetic integrity of each population, cross pollination between different accessions is prevented.

Self pollination in some species(pepper, tomato, soybean, mungbean) ensures that cultivars and landraces are quite uniform. However, varying degrees of natural cross pollination has been known to occur, even in prodominantly self-pollinated species. This can be as high as 91% in pepper (Tanksley 1984) and 0.2 to 47% in eggplants (Quagliotti, 1979). The degree of cross pollination is influenced by distance of plants, wind direction and insect activity with the last contributing the most to cross pollination (Murthy and Murthy, 1962: Francheschetti, 1972). Therefore, peppers and eggplants are multiplied under insect-proof nylon net cages to prevent the entry of insects that may cause cross pollination. A row between plots is left vacant to separate accessions. Furthermore, plants are staked and pruned if necessary to prevent intermingling of branches. These procedures also minimize the possibility of mixing fruits harvested from adjacent plost.

Selfing can be greatly increased by hand pollination but it is too tedious and therefore, resorted to only in special cases, e.g. low seed producers.

For naturally cross-pollinating species like onion and *Brassica,* each accession is enclosed in one net cage. Insect pollinators are released inside the cage to enhance pollination.

The use of net cages has posed several problems. Aside from being expensive and laborious to construct, the shading that results affects the growth of the plants and may distort characterization data. The environment inside the net cage is also so favorable to mites that severe infestation is frequent and often difficult to control even with repeated spraying of miticides. On the other hand, growing in net cages results in the exclusion of insects that can cause damage to the fruits, e.g. fruit borer that would cause reduced seed yields as well as reduced seed vigor (Krishnasamy, 1990).

Our national partners also practice controlled pollination in okra, *Momordica* and *Cucumis,*

Some accessions show a great amount of phenotypic variation and although other accessions may look uniform based on morpho-agronomic traits, they may carry hidden heterogeneity (Quagliotti et al., 1972: Pickersgill, 1986). In pepper, our data show that out of 2,118 accessions characterized 60% can be considered as homogeneous and only 6% as highly heterogeneous. An inherently heterogeneous population is harvested as a bulk while populations where distinct variants can be recognized are divided into sub-accessions.

Characterization

Systematic characterization using morpho-agronomic characteristics begin with the first seed increase. Such characterization data not only provide information on the potential utility of the accession but serve as identifying information that can be used to check on the correct identity of the accession during regeneration. In addition characterization data can be used to evaluate the extent of diversity in the collection and identify potential duplicates. Characterization is usually done based on the IBPGR set of descriptors. Morpho-agronomic characterization is now complemented with biochemical characterization (e.g. capsaicin and sugar content in pepper, protein and starch in mungbean and SDS-PAGE of seed proteins).

As in collecting and seed increase, AVRDC has sought the collaboration of NARS. To strengthen cooperation, training of nationals in vegetable germlasm management became an integral part of the collaborative projects.

Preservation of Germplasm

The preservation of assembled germplasm is the core of genetic conservation (Chang, 1985). For vegetable species with orthodox seed, seed preservation is the most efficient means of maintaining large numbers of accessions and making them available for distribution.

The goal in seed preservation is to maintain genetic integrity of the population and to maximize the longevity of the stored seeds at minimal cost. Longevity is affected by two factors: the status of the seeds before entry into storage and the conditions surrounding the seeds during storage.

Processing and handling seeds for preservation. The aim of preservation is maintenance of high seed viability. High viability not only helps maximize the possibility of an adequate number of healthy vigorous seedlings being established, but also minimizes the risk of genetic damage that have been observed in several instances (Ellis, 1991) Many national genebanks consider loss of seed viability during storage one of their most serious problems, even though some of them have good storage facilities. It appears that improper pre-storage processing may be the cause of such rapid loss of viability (Engle and Chang 1991).

Precautions during processing and handling are not only meant to maintain high viability but also to avoid mechanical mixtures.

Harvesting. Harvested fruits are transported and dried in net bags to minimize accidental mixing of different accessions. Each accession is processed separately from the others.

One of the factors that affect longevity of stored seeds is the quality at harvest. Seed should therefore be harvested at stage of highest quality Reccently, experiments by Demir and Ellis (1992)contradict the widely accepted hypothesis of Harrington (1972) that a seed attains its highest quality (i.e., viability and vigor and therefore storability) at physiological maturity and that deterioration as a result of aging starts thereafter. Their experiments showed that in *Capsicum annuum,* maximum potential longevity was achieved 63 to 65 days after anthesis. This is 10 to 12 days affter mass maturity that they defined as the end of the seed-filling phase. Similar results in tomato led them to conclude that delayed harvesting results in little or reduction in seed quality in those seed crops in which seed m.c. is maintained naturally within fruits at values approaching those of fully imbibed seeds.

Other studies show that in both pepper and eggplant, better quality seed is obtained from a slightly advanced harvest than from a delayed harvest. For "Tabasco" type pepper (*C. frutescens*), germination was greater and faster in red fruits harvested early than in immature orange fruits or those harvested post red mature in the growing season (Edwards and Sundstrom, 1986). Some seed producers leave the fruit on the plant until the abscission layer just behind the calyx is fully developed. However, over-ripe fruits should be avoided. In domesticated peppers which lack dormancy, the seeds may germinate within the moist interior of the fruit while it is still on the mother plant Such pregerminated seeds will be inviable when dried for storage (Pickersgill, 1986).

In eggplant, the germinability of seed occurs 40 days post anthesis

on the plant and increased germinability can be observed after 20 days post harvest ripening at room temperature applied to the fruit harvested 40 days after anthesis. When 100 seeds fresh and dry weight approach maximum values, germination rises abruptly from below 10% to above 80% within a very short period (Osman, 1989).

Leaving the seeds in the fruit for month after harvesting before removing them greatly increases the germination percentage in pepper although 1000 seed weight remains practically unchanged. The minimum post-harvest ripening time that can be considered effective is 20 days. On the other hand, it is worth prolonging the post-harvest ripening period to 30 days (Quagliotti, 1977). In 'Tabasco' pepper optimum post-ripening occurred after 21 days at 25°C (Edwards and Sundstrom, 1986).

Seeds for preservation should be free from injury, surface infestation by microbes, or insect infestation. In addition, they should be harvested from plants that have not suffered stress due to water deficit, water excess, or nutritional deficiency in the field.

Seed-borne mycoflora has been described in several publications. Among seed-borne mycolflora, *Fusarium moniliforme* caused 6 to 8% loss in germination of pepper seeds (Hashmi, 1989). Pepper seed infected with *Colletotrichum* spp. often has poor germination or viability (Grover and Bansal, 1970).

Seed extraction. For large-fruited and low yielding accessions of pepper, eggplant and tomato, the fruits are usually split open with a knife the seeds extracted manually.

Hot peppers cause a big problem during extraction. Capsaicinoids can irritate the hands of those who do the extraction manually and indirectly the eyes, nose, and other sensitive tissues of the body. Dried fruits when processed produce highly irritating fine particles. Even fresh fruits in large quantities release fumes that irritate the lining of the nose and cause watery eyes. Many workers find it uncomfortable to wear gloves and masks when extracting seeds. They prefer to work outside the building or to use fans that can minimize the discomfort caused by the strong fumes. For sensitive hands, we have tried dipping hands in starch milk and soapy water after work. Starch seems to alleviate the discomfort. A mild solution of Chlorox has also been suggested (Poulos, personal communication)but is also harsh on sensitive skin. Doing the extraction inside table top cabinets with fume absorbers may help.

For small-fruited and high yielding accessions of pepper, a meat grinder can be used to cut the fruits into small pieces and the seeds

collected by water extraction (Tay, 1989.) Alternatively, the fruits inside the net bags can be threshed in water by trampling. The seeds are then washed clean of the accompanying pulp. During washing underdeveloped seeds usually float and can be removed.

Eggplant fruits are softened by beating or rolling for easier extraction. Seeds can then be manually separated from the pulp and washed clean with water.

At every step, care is exercised to minimize deterioration and mechanical damage to the seeds or mixtures.

Drying seeds. seed moisture content (m.c.) is one of the principal factors in determining seed longevity Lowering the moisture content of seeds result in improved seed longevity (Zhang and Tao, 1989; Ellis et al., 1989, 1990: Vertucci and Roos, 1990).

A three to seven percent moisture content depending on species is now recommended (IBPGR, 1993)

Emphasis on lowering seed m.c. to below 10% and the availability of free solar energy has prompted many to sun-dry or to use mechanical drying temperatures that exceed 40 ℃ (Engle and Chang, 1991). This could lower longevity of seeds. The use of heat at 35 to 45 ℃ is known to result in seed deterioration, in some vegetable seeds (Tao, 1988). Experience at AVRDC reveals that temperature of cement floors exposed to the sun can attain temperatures of 42 ℃ even on a cool-dry day (min 14.6 ℃) 48% RH, max 25 ℃ , 98% RH ambient conditions). IBPGR recommends that seeds are best dried in a drying room maintained at 15 ℃ and 10 to 15% RH with good circulation. At AVRDC, this condition is attained with the use of a sorption type air dehumidifier with refrigeration to lower the temperature and remove heat generated by the air dehumidifier. The seeds in net bags are placed in open trays inside the drying room for two weeks to dry down to at least 8% m.c.

More recently drying to less than three percent is recommended for some oily seeds (Ellis et al., 1990). It is not known if such ultra dried condition would provide added benefit to the preservation of pepper of eggplant seeds.

Another method is to use silica gel for drying. Seeds are placed with silica gel in a closed container. The seeds are kept in porous bags to separate them from the silica gel. Depending on species, seed m.c. can be lowered to three to seven percent within a month. A 1:1 seed to silica gel ratio (by weight) is generally used (Zhang and Tao, 1989).

Freeze-drying has been reported as another method to lower seed

moisture to the two to five percent range by avoiding potential damage due to heat and over desiccation. Seed storability at warm temperatures may be improved by this technique (Woodstock et al., 1983).

Packaging. Packaging is done to keep each accession separate, to prevent contamination of the seeds from insects and diseases and to minimize absorption of water by the dried seeds.

We find the use of aluminum polyethylene envelopes (AL-PE) convenient for both the base and active collection. Seeds for distribution are packed in AL-PE envelopes in 50-seed quantities. This allows the retrieval of a specified amount of seeds of each accession without opening and closing containers. It also provides protection from absorption of moisture by the seeds due to inadequate dehumidification of the cold store and during transport.

Storage conditions. In all its undertakings, GRSU strives to meet scientifically designed standards and recommendations set by IBPGR.

As a guide, the Preferred Standard for long-term seed storage-18℃ (Cromarty et al., 1985) is used.

For medium-term storage the temperature ranges from 0 to 10℃, RH from 15 to 50%, and seeds are stored in fairly airtight containers. The projected seed longevity ranges from 10 to 30 years or longer. It is a common experience that the required temperature is easy to attain if RH is not considered. However, maintenance of a constant 45% RH in cold stores is a big headache especially in the tropics. Shifting to airtight containers such as aluminum foil bags will allow working within a narrow range of temperature and a wider range of RH. The 1993 proposal is for storage conditions to be such that viability of accessions remains above 65% for 10 -20 years (IBPGR, 1993).

Recently, Benson (1990) assessed the rold of oxidative stress and free radical-mediated damage in the safe conservation of plant genetic resources as well as the possible effects of anti-oxidants and recovery/ repair mechanisms.

Preservation *in-vitro* and in field genebanks. We are fortunate that most vegetables have orthodox seeds and only a few of them have to be multiplied by vegetative means. Among the principal crops of AVRDC, the *Alliums* need to be preserved *in-vitro* and in field genebanks.

Monitoring

To ensure that seed storage conditions are maintained at desired

levels, regular monitoring is required. Seed m.c. and viability need to be tested before sealing and over time during storage. Temperature and RH in the storage rooms need to be monitored as well. The amount of seed in store should be monitored especially for active collections that are used for distribution purposes. The amount of seed in store and seed viability are two factors that are considerd in deciding when regeneration of seed lots should be done.

Roberts (1975) has suggested that when seed viability drops to 85%, regeneration is warranted. However, for some crops, high initial seed viability is difficult to attain. For example initial viability testing in pepper has shown that only about 75% of the accessions tested have initial percentage germination higher than 85 (AVRDC, 1992). The consideration of this fact in the new recommended standard for genebanks (IBPGR, 1993) is most welcome. Regeneration is now recommended when viability falls to 85% of the initial value.

Regeneration and viability monitoring are two of the most laborious, time consuming and expensive activities in germplasm conservation. Regeneration becomes formidable when the collection is a large one. In the case of viability, monitoring methods used routinely are destructive so seeds that have been painstakingly multiplied have to be sacrificed during this activity. More practical approaches such as the adoption of the core collection concept (Frankel and Brown, 1984) during regeneration and the use of check varieties or clusters (AVRDC, 1992) in monitoring viability may have to be considered. The use of non-destructive methods is another possibility.

Enhancing Utilization

It has been repeatedly said that evaluation and utilization have not kept pace with the collection activities: in Southeast Asia by the Regional Committee for Southeast Asia or RECSEA (Singh and Chomchalow, 1984).

AVRDC recognizes that the conservation, enhancement and wide distribution of plant germplasm is one domain where the comparative advantage of international centers is most profound and, probably most lasting. AVRDC therefore sees as one of its key global roles the conservation and free movement of vegetable germplasm to whoever can make good use of them. The center sees itself as a steward for such genetic resources and commits itself to their free and unrestricted distribution (AVRDC's 5-year Plan).

To enhance utilization, it is necessary to have a large active collection for distribution purposes. The AVRDC tomato collection has already been described as the largest and most comprehensive active collection of tomato germplasm in the world. GRSU's goal is to complement its base collection with a dynamic active collection.

In addition to having enough seeds for distribution, utilization can further be encouraged if evaluation data is available. Morpho-agronomic characterization is usually routinely done by genebank staff during multiplication for long-term storage. On the other hand, evaluation for traits of immediate importance to breeders (e.g. resistance to pests, diseases and abiotic stresses, improved quality, etc.) are best done with the participation of researchers from several disciplines (e.g. entomologists, pathologists, virologists, physiologists, chemists, etc.). In this way, invaluable genepools that can be channeled into breeding programs will be identified.

Safeguarding Seed Health

An AVRDC Seed Health Committee oversees the formulation and implementation of appropriate seed health practices in the germplasm and seed resources unit. Close interaction with the host country's quarantine authorities ensure that our practices meet and the national phytosanitary standards (AVRDC, 1992).

Newly collected materials are planted in post-entry quarantine screenhouses. And for vegetatively propagated materials, the policy is to distribute *in-vitro* materials that have undergone a virus elimination and virus indexing procedure.

The Challenge of the Future

New technologies are fast emerging. Techniques in molecular biology are now finding application in the management of germplasm. RAPD and RFLP will soon be routinely used in accession identification and characterization of genetic diversity in populations. Hopefully, they can also anwer some of the questions regarding the occurrence of genetic drift and shifts in genebanks. Statistical procedures are being used to make the task of the genebank more manageable as in the formation of core collections. Computers are cheaper, more powerful and easily available even in developing countries.

But even while such new technologies are attracting attention,

classical seed physiology studies still need to be done on some crop species, Non-destructive procedures for viability determination still need to be established.

Conclusion

From the above it can be seen that the management of vegetable germplasm for use in preservation and distribution encompasses a web of complex interrelated activities. Many of the procedures and conditions followed are based on general precepts on preservation of germplasm. Only a few are based on actual research and data obtained.

The stringent requirements for preservation of germplasm necessitate the availability of facilities and funds designed for long-term operation as well as trained and dedicated personnel.

Genetic erosion due to loss of viability can be serious in the maintenance and storage of germplasm. Such loss is demoralizing after all the effort, risk, and cost of launching a well-planned collecting expedition, and documentation of the collections. Appropriate handling, storage procedures and management are necessary to preserve the genetic diversity gained through collection.

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GERMPLASM RESOURCES INFORMATION NETWORK (GRIN) — A Case Study for Designing a National Plant Germplasm Information System.

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ABSTRACT

The Germplasm Resources Information Network - Database Management Unit has been in operation for ten years. During this time, technology for managing information on genetic resources has improved and the demands for the information have increased. Many of the lessons learned by the Unit are relevant to germplasm programs in other countries and for other organisms. The most important lessons being: to maintain good communication between the system developers and the users; to keep the system flexible and adaptable to change; and to follow international standards whenever possible. The Database Management Unit is designing the third version of the software now and the system will continue to evolve as improvements in technology make it possible to manage more information, answer more questions, and communicate with other systems around the world.

INTRODUCTION

Ten years ago the Agricultural Research Service (ARS) created the Germplasm Resources Information Network-Database Management Unit (GRIN-DBMU) to complete and manage the information system for the United States National Plant Germplasm System (NPGS). Many changes have occurred within the NPGS since then and the challenge for the GRIN-DBMU has been to develop a flexible system and anticipate the needs of scientists and curators. The history of this system and the GRIN-

DBMU is detailed elsewhere (Mowder and Stoner, 1989). The purpose of this paper is to examine the influence of technology and other key factors on the decisions in building the system.

The goal of the GRIN-DBMU has always been to create a centralized information system to support a distributed repository system. Currently, there are four Regional Plant Introduction Stations, six special crop collections, eight clonal repositories, a base storage facility and five supporting sites distributed across the United States from Geneva, New York to Hilo, Hawaii, maintaining information on a wide diversity of genetic resources.

Information is accessible from the computer seven days a week and 365 days a year except when the database is off-line for maintenance and back-up.

TECHNOLOGY: Then and Now

At the time that the first version of GRIN was designed and developed (before 1984), information technology was very different from today. Very few of the repositories had a microcomputer because they were expensive and unproven.

Microsoft DOS was at version 2 and competing with CP/M and Apple DOS for marketshare. Telecommunication equipment for the repositories was being purchased with a speed of 120 characters per second (1200 bps) over the TELENET X.25 packet-switched network or synchronous dial-up modems, barely able to keep up with a good typist. Research scientists could reach the computer through a rotary of 300 and 1200 bps dial-up modems.

Curators and support staff had limited computer knowledge, usually confined to one particular machine and operating system which was different from site to site. The National Seed Storage Laboratory used a UNIVAC at Fort Collins Computer Center, the Southern Regional Plant Introduction Station at Griffin, Georgia used an IBM mainframe, and the Plant Introduction Office used a Datapoint computer. There was no electronic communication between these machines, all information was passed from site to site in printed form. Computer storage of information was expensive and the information was typically stored as short, cryptic codes decipherable only by the staff at that site.

Database management systems (DBMS) for production systems were either network or hierarchical, such as IDMS or IMS. Relational DBMS

were in their infancy and generally limited to small or experimental systems. The ARS had recently purchased a Prime model 750 minicomputer for the GRIN system which was rated at one million instructions per second (MIPS) and had 1200 megabytes (MB) of disk space. The GRIN software was being developed in FORTRAN with function calls to the Prime DBMS, a CODASYL standard network database. Each site was given a single terminal and printer to access the database.

Today, microcomputers have replaced terminals as the interface to the system. Nearly every scientist and staff person handling information in the NPGS has a microcomputer on their desk or will have one in the near future. Most scientists are computer literate, write manuscripts with their word processor, manage data sets with a file management system (e.g. dBASE, Foxpro, Paradox) or an electronic spreadsheet program (e.g. Lotus).

Microsoft is now shipping version 6 of its operating systems and dominates the operating system market. Computer storage of information and processing power are more affordable, evidenced by the introduction of gigabyte (1,000 MB) disk drives for a microcomputer and an Intel 80486 processor rated at 16-20 MIPS.

Relational DBMSs have become the standard for large, multi-user production databases and object-oriented DBMSs are now the experimental systems. Third-generation coding languages such as FORTRAN are being replaced with fourth-generation forms packages and computer aided software engineering (CASE) tools. The GRIN-DBMU is currently developing a new version of GRIN in a relational DBMS to run under UNIX on a multi-processor minicomputer.

Telecommunication speeds and methods also have changed. Most locations have 9600 bps access over FTS2000 X.25 packet-switched service and we have just installed the first 56,000 bps access to a remote site. Dial -up access is now available at 19,200 bps using PEP technology or 9600 bps with MNP5/v. 42bis data compression. The computers at repositories are being connected togeter as local area networks (LAN) and these LANs connected into wide area networks. Currently, ten sites have installed LANs or are in the process of doing so.

The new and old GRIN central computers are also networked together and connected to the Internet which is a network of networks reaching to more than 100,000 computers around the world. A gopher server has been set up on the new computer to access the information of the National Genetics Resources Program of ARS. Eventually this

computer will have database of plant, animal, microbial germplasm maintained within the United States Department of Agriculture.

The following trends should continue for the foreseeable future:

1. Faster computers storing more information.
2. More rapid system development with more powerful tools.
3. Increasing connectivity (i.e. more networks and inter-networking) and faster communication speeds.

The first two trends will have their impact on the building and managing national information systems while the last trend well have its impact on sharing the information with the international community.

IMPACT OF CHANGING TECHNOLOGY ON GRIN

This changing technology has had several impacts on the GRIN. First, pre-GRIN databases started with simple models and data structures with the trend to increase complexity.

For example, examine how germplasm origin as a data structure has evolved in the GRIN system. In the GRIP Design Recommendation Report in 1981, only the country of origin was identified as a data structure for origin. In the first production version of the system, GRIN1, the origin state/province field was added to the origin record. This data is very important when a country is divided after a war or for other political reasons. Latitude and longitude fields were added to the accession record in GRIN2 so there is less question of the sample's origin when political boundaries change. Previously, this information was stored in narrative, and some still is, which is of little use except when manually searching the records.

Germplasm is increasingly being exchanged between gene banks and furthering the need for tracking more information about the origin and history of the material. Enhancements to GRIN2 included fields to the accession record for the history of the germplasm before its acquisition by the NPGS and who developed or collected the material.

The design of the new version of GRIN, GRIN3, calls for moving the whole concept of origin out of the accession record so we can note all of the places and people that may have been associated with an accession prior to acquisiton. This information is going to become increasingly important for determining the overlap between gene banks of the world as well as intellectual property rights and the Convention on Bio-diversity.

Another example of the increasing complexity of the data being

maintained can be found in the evaluation area. Initially, it was viewed as a limited set of descriptors, collected in a single environment and variability within the data was ignored or treated in a simplistic way. The curator could choose to store their data as a range (minimum and maximum) or as the mean of a set of observations. In the GRIN2 version, we introduced the single-observation record which allowed linking each observed value for an evaluation descriptor of an accession to a different environment and the number of descriptors for a crop did not need to be predefined. Instead, as additional descriptors were identified and evaluations conducted, the result could be added to the database without changing any software. The structure for a qualifier of the descriptor also was added. The qualifier field has been used for identifying the race of pathogen used in a particular evaluation. For example, wheat has been evaluated for Hessian fly (*Mayetiola destructor*) resistance of different biotypes or isolates (e.g. H3, H6, 'Great Plains') and it would not be desirable to treat each test on each strain as a separate descriptor. With the biotype stored in the qualifier field, it is possible to search the database for Hessian fly resistance or just those accessions resistant to the Great Plains isolate of Hessian fly.

GRIN3 will add even more information about an observation. The new database has fields for the minimum value, maximum value, standard deviation and sample size which will provide more information on the reliability of the observed value.

Future versions of the system will likely contain other kinds of data about the accession, including images of the plants, seeds, and fruiting structures. However, this has to wait for further developments in disk storage, telecommunications, and software. Disk storage needs to be less expensive and have higher transfer rates. A high quality image takes several megabytes of space and we have more than 400,000 accessions which may require several images. It now takes 3.5 minutes to send a megabyte image at 56,000 bps. Once the image is sent to a user, standards for displaying and manipulating images with common telecommunication software need to be implemented. Data compression techniques can reduce the size of the image files and shorten the transmission time but the software at the receiving and must also be compatible.

ROLE OF THE USERS

The key to the success of the GRIN system has been user involve-

ment. Continual dialogue between the DBMU and the users at the repositories during the development and enhancement of the system through telephone conversations, electronic mail and regular meetings have been used to gather input from the sites, resolve conflicting requests between sites, and educate the staff at the sites on how to use the software. Involving these people has also been important in making them aware that it is their system and their data. It is easy for the personnel to give a remote system lower priority and find work to do locally. The system has to have clear benefits to each and every user either by making them more productive or the work less tedious.

ROLE OF STANDARDS

Standards are important to genetic resources information systems for several reasons. First, to convey information from one person to another. If the Plant Introduction Office and repositories use different sets of codes for names for countries then the data must be converted to be useful. If both the Plant Introduction Office and the repositories agree to an international standard like ISO country names and alpha-2 or alpha-3 codes, not only should they be able to understand each other but the information should also be acceptable to other gene banks around the world.

The use of standards also reduces the amount of overhead that must be passed with the data. If two locations agree on a standard list of country names and codes then the lists do not have to be exchanged every time the accession data is passed between the users.

Finally, the standards help with the maintenance of the data. When a new introduction record is added, the standard list of names can be used to verify the spelling and thus reduce errors at the time of data entry.

The GRIN-DBMU continues to monitor several standards setting organizations which may impact the data and the date structures of GRIN. The following are some examples:

- Interantional Standards Organization. (ISO)-currently working on a standard list of states and provenances.
- International Organinzation for Plant Information (IOPI)-working on a standard list of scientific names for name and data structures to hold taxonomic, economic, and geograpical information.
- International Board of Plant Genetic Resources. (IBPGR)-developing crop-specific descriptors, standard systems for handling genetic

resources, and coordination of international base collections.

- American National Standards Institute (ANSI)-continuing to develop standards for computer languages, particularly C, C++, and SQL.
- National Center for Biotechnology Information (NCBI)-Promoting the use of Abstract Syntax Notation (ASN.1) for the exchange of date electronically.

The users of the system are surveyed regularly to determine what technologies have been implemented and become the de facto standards for such things as hardware including printers and modems; telecommunication and database software; and LAN configurations and operating system.

THE PAINFUL LESSONS.

During the ten years that the GRIN system has been operational the GRIN-DBMU has learned many lessons, some more painfully than others. The first lesson was to load good, accurate data and take the time to thoroughly check it for consistency. The initial loading of the GRIN database took data from a wide variety of systems operating at the repositories and support sites. There were problems both with the consistency of the data and the software used to load the data into the database. Since the data was being maintained independently, transposition errors in the accession identifiers were not detected until two sites claimed the same accession. Even worse problems were errors created by the loading programs because a large number of records were effected.

The second lesson was to take the time to test and check the software. The second version of the software, GRIN2 had over 250,000 lines of FORTRAN code and reached the users without much quality control. The users became very frustrated because of the problems encountered and the software aborting their work.

Another lesson was that once the users accept the system and rely on its use, it is extremely important to keep the system reliable and functional. Disaster recover and contingency plans need to be reviewed and updated regularly. Twice the GRIN system has been down for an extended period of time. Once for the electrical re-wiring of the computer room due to an uninformed building electrician's knowledge of what is required to ground the high frequency feedback from a computer. The second instance was due to internal inconsistencies in the database that went undetected

for several months and eventually required the unloading and reloading of the entire system, which itself took more than a month. In both cases, repositories that had been using the system for their day-to-day operations were forced to revert back to manual record keeping and then update the database when it came back on-line. The extra work and the delays caused by the manual processing of the data reduced the productivity of the staff, increased their frustration, and gave them a sense of not being in control of their operation.

SOME OTHER VALUABLE LESSONS LEARNED

Keep communication lines open with the staff at the repositories through as many means as possible. The more informed and educated the users are about the system, the better they respond to questions about what they would like changed and improved.

Try to anticipate changes in the way the users want to use the system and the information they wish to store. As users added more data to the system and used it for maintenance of their inventories, the GRIN-DBMU received requests to add additional fields and procedures to manage the information. For example, when seed is sent to NSSL for long-term storage, they wished to record the site inventory sample identifier as well as the accession identifier of the material. Other fields were added for recording when an accession was placed in quarantine, where it was held in quarantine, and when it was removed from quarantine.

Another valuable lesson that was learned is to spend time understanding how the software works and how the data is managed at a very technical level for the purpose of tuning and correcting problems. Several times in the last ten years the GRIN-DBMU has had to use a binary editor on the files in the DBMS to correct pointers, patch b-trees in indexes, and reconstruct data. On a three gigabyte database it takes a large amount of the resources of the computer to monitor and protect the integrity of the data. A program to validate all of the links for all the inventory records in the current system, about 700,000 records, takes a month to complete.

Finally, as a system grows in size, it takes people with many different talents and specialties to manage the system. The GRIN-DBMU currently employs 14 people including:

- Database manager to supervise the group.
- Secretary.

- System administrator to oversee the hardware maintenance and computer room operations.
- Database administrator to monitor the database and lead the development of new software.
- LAN administrator to manage the Laboratory LAN and act as back -up to the system administrator and database administrator.
- Three software developers for writing new software and maintaining existing code.
- Data administrator to manage the loading of data and developing rules for how the data should interrelate.
- Crop Advisory Committee facilitator to keep good communications with the users of the system, both managers of the information and their industrial counterparts who guide our efforts.
- Three other people that work with data, either entering data or managing critical parts of the system, such as the standards area or intellectual property rights, such as patents and cultivar registrations.
- Technical document specialist for producing manuals and other documentation for the system.

The GRIN system would not be where it is today, if it wasn't for the contributions by all of the individuals in the GRIN-DBMU working as a team. Members of the unit also perform other roles including software testing, technical evaluation and equipment purchase, generating reports for management, and formatting data for distribution.

As the computer industry continues to change, the GRIN-DBMU needs to develop additional expertise in other areas. Most of the emphasis and expertise is on plant germplasm and a central, multi-user database. With the 1990 Farm Bill, the role of the GRIN-DBMU was expanded to include other agriculturally important germplasm (microbial, livestock, poultry, fish, forest trees, etc). Microcomputers software continues to change and expertise is needed to update existing software (eg. pcGRIN) and write new software to take advantage of the features such as windowing environments, database front-end tools, and multi-user LAN applications. Finally, expertise is needed in the GRIN-DBMU to link our databases with those in other countries and other kinds of computers. This requires knowledge of other scientific disciplines, international telecommu-

nications and, possibly foreign languages.

Disclaimer: Brand names of computer hardware and software are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Mowder, J. D. and A. K. Stoner. 1989. Information systems. p. 57-65. In: J. Janick (ed.) 1989. Plant Breeding Reviews. Volume 7. The National Plant Germplasm System of the United States. Timber Press, Inc., Portland, Oregon.

Appendix I

GRIN System

Growth in the Database

1983	--	300 MB allocdted
1985	--	900 MB allocdted
1988	--	1,800 MB allocdted
1992	--	3,400 MB allocdted

Source History Example

PI 511511 *Zea mays* (field corn)

- Collected in Brazil
- Donated by CIMMYT in Mexico
- Received through Pioneer Hi-Bred

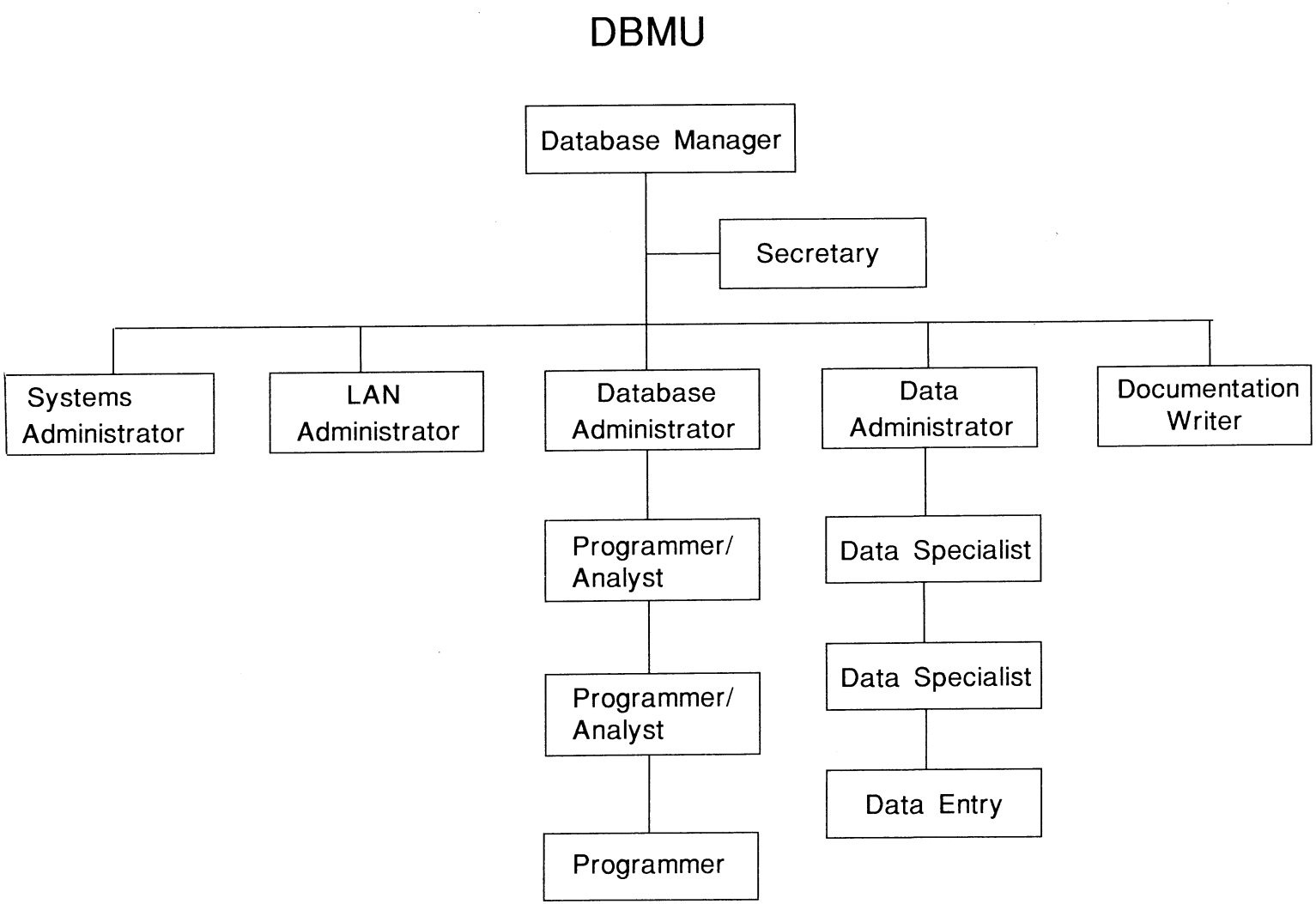
GRIN System

Resistance to Hessian Fly, Biotype' Great Plains'

Accession ID	Resistance
PI 68284	2
PI 243787	1
PI 320193	1
PI 324928	1

(select WHEAT Hessian-Fly $<$ 4 and DSQUAL = 'BIOTYPE-GP')

Appendix II Organizational Structure of the DBMU



Plant Germplasm Conservation: Perspectives for the 2000s. 61-81 (1994). Taiwan Agr. Res. Institute

RECENT ADVANCES IN SEED PRESERVATION TECHNIQUES

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ABSTRACT

Although seed longevity and storage have been of interest to humankinsd since the beginning of agriculture, the systematic collection and long-term preservation of seed germplasm is only a relatively recent practice of the 20th century. Seed longevity claims of thousands of years have not been verified by the scientific community However, seed viabilities in the range of a few hundred years have been well documented. Even some vegetable seeds, which have been thought to survive only a few decades, have recently been shown to germinate after more than 50 years of storage. The search for physiological, biochemical, and/or biophysical mechanisms of seed deterioration has begun to shed new light on how seeds lose their vigor and germinability. Some studies of particular interest have focused on the integrity of cell and organelle membranes, the fragility of DNA and chromosomes, and more recently the role of water in controling longevity at various temperatures. In the case of membrane integrity, we have learned that membranes undergo phase transitions between the gel and liquid crystalline configurations, depending upon the moisture content; and, that this can be controlled by temperature. The genetic integrity of a seed or a population of seeds can be altered considerably during seed aging and deterioration, thus resulting in loss of variability or genetic shifts in populations. A better understanding of the interrelationships between seed moisture content and storage temperature has just recently shed new light on the potential longevity of orthodox seeds. This understanding of deterioration may lead to the rescue of aged seeds and the retrieval of valuable genes once thoughtlost. In addition to the factors of moisture content and temperature, the seed developmental stage, is now known to be vitally important in the preservationof recalcitrant seeds. In

this day, modern genebanks cannot limit themselves to orthodox seed preservation alone.

INTRODUCTION

I am indeed proud and honored to have been invited to participate in the dedication of the new National Plant Genetic Resources Center here in Taichung. This new genebank is certainly one of the most modern and beautiful buildings in the world dedicated to the preservation of plant germplasm. The Council of Agriculture was wise in choosing to locate this Center here on the campus of the Taiwan Agricultural Research Institute as it provides the leadership and research atmosphere to insure a strong and fruitful program in acquiring and preserving your valuable plant genetic resources. I congratulate you all and wish you the greatest success in the future.

In discussing recent advances in plant germplasm preservation, I would like to review current information on seed longevity, deteriouation, cryopreservation, storage protocols, image analysis techniques, seed rescue and include a brief discussion on preservation of non-seed germplasm. Much of this work is being done at the National Seed Storage Laboratory in Fort Collins, Colorado and i hope this presentation will provide stimulus for scientific exchange and cooperation between our laboratory and this new genebank in Taiwan.

SEED LONGEVITY

We can only imagine the great excitement generated by the *Gardener's Chronicle* article which appeared November 11, 1843, describing the germination of 3000 year old wheat seeds, presumably taken from a tomb in Egypt (Anonymous, 1843; Priestley, 1986). Such claims of extreme longevity have appeared frequently in the popular press without any scientific proof of authenticity (see review by Roos, 1986, 1989). The longest claimed longevity was for seeds of *Lupinus arcticus* (Table 1) which were said to have still germinated after 10,000+years buried in the frozen tundra of Alaska (Porsild et al., 1967). However, as with the 'mummy wheat' seeds, no compelling scientific supporting evidence was presented to document the claim. The subject of seed longevity is a fascinating one and it becomes more intriguing when results of actual experiments are examined, such as the reports of the Beal buried seed experiment (Kivilaan

and Bandurski, 1981) in which weed seeds buried in the soil were dug up after 100 years and found to still have some viability.

Longevity experiments: I have been fortunate to have become involved with two long-term storage experiments. The first involves a continuation of a vegetable seed longevity study first reported on by James et al. (1964). Seeds of 15 vegetable seeds were germinated after storage for 15 to 30 years in an office in Cheyenne, Wyoming. Retesting some of these same lots 29 years later showed that some viability was still present after 50 to 60 years (Roos and Davidson, 1992). the greatest reported longevity for most of these species (Table 2).

The second experiment, initiated by Went and Munz (1949). concerend storage of 98 species and varieties of California desert plants. Recently I was able to obtain the actual residual seeds of this experiment from the Rancho Santa Ana Botanical Garden in San Diego, California where they have been stored unnoticed for several years. These seeds, vacuum sealed in glass vials, have been transferred to the National Seed Storage Laboratory and will be tested in the coming year. Of some significance is that, to my knowledge, they have never been stored under sub-freezing conditions.

The advent of modern seed genebanks, such as the one here in Taiwan, present the interesting challenge to preserve our seed genetic resources indefinitely, under optimal conditions as a safeguard for the future of all peoples of the world. The knowledge of the past will be enhanced and transferred to future generations.

SEED DETERIORATION RESEARCH

Several theories have been advanced to explain why seeds deteriorate, even under relatively good storage conditions. These have been discussed previously (Roos, 1989) and are summarized here.

Depletion of Food Reserves: This theory suggests that over time essential substrates become depleted. The major food reserves (storage carbohydrates, proteins, and lipids) may show minor losses over time as a result of respiration. However, examination of non-viable seeds shows that plenty of reserve food remains. Also, we now know that seeds stored at low moisture contents(less than 8%) have essentially no mitochondrail activity. Thus, depletion of food reserves by mitochondrial path ways is likely minimal. Loss of enzyme cofactors (vitamins and minerals) has not been linked with loss of viability.

Alteration of Chemical Composition: Although food reserves may not be depleted, they may be altered chemically so that they are not in a usable form. For example, Crocker and Groves (1915) suggested that seed death resulted from a "coagulation of protein." Also it is well known that lipids undergo oxidation, and an increase in fat acidity has been associated with deterioration. Proteins also underge changes as seen by decreased solubility, partial breakdown, and decreased digestibility. Many other compounds may change quantitatively or qualitatively during storage.

Membrane Alteration: The theory that membrane alteration causes deterioration has its basis in the observation that aged seeds have a great propensity to lose cell constituents upon imbibition. This "leakiness" is attributed to a loss of membrane integrity. Although the exact mechanism causing the membrane alteration is not known, speculation surrounds the role of peroxidation of unsaturated fatty acids. In essence this theory could be considered a corollary to the previous theory. Much of the current research on seed deterioration is concentrated on examining membrane alteration.

Enzyme Alteration: Duvel (1902), in his Ph.D. dissertation, and later White (1909) proposed that respiration and enzyme activity were important in the preservation of vitality. Many enzymes have been studied in an attempt to correlate seed viability with the amount of enzyme activity. The basic problem with regard to loss of enzyme activity seems to be that of cause and effect. Is loss of enzyme activity the cause of lost viability or is there another cause which also affects loss of enzyme activity?

Genetic Damage: Alteration of the cell's DNA, such that it is unable to duplicate and divide, could occur from either chemical mutagenesis or from ionizing radiation. It is a well-established phenomenon that deteriorating seeds produce an abundance of chromosomal aberrations as seen in root tips of germinating seeds. Seed viability has been inversely correlated with the frequency of these aberrations. Again, the mechanism for the production of the aberrations is not known. However, evidence has been advanced that the increase in chromosomal aberrations with aging, may be a result of the breakdown in the cell's natural DNA repair mechanism(s).

Research Approaches: Current research has focussed on the last three theories and I would like to present some additional thoughts on each of these, beginning with genetic changes and their potential importance for germplasm preservation. Basically there are two topics of concern. The

first involves potential damage to the DNA itself and is reflected in mutations, chromosomal aberrations and failure of replication, and/or transcription and translation of the genetic code. Most of the recent research has concentrated on the phenomenon of chromosomal aberrations, as seen in the root tips of germinating seeds. Aged seeds have long been known to produce these aberrations (Barton, 1961; Roos, 1982); however, newer research (Murata et al., 1984) has shown that these abnormal cells do not pass on the aberrations to the next generation of seeds (Fig. 1). Thus, it appears that germplasm accessions are not subject to an accumulation of these changes over successive generations.

The other genetic concern involves the potential for genetic shifts to occur in heterogeneous germplasm populations, typical for many of our landraces and wild populations. Some of my research (Roos, 1984a,b) on mixed bean populations (Fig. 2) demonstrated how rapidly such shifts can occur as a result of differential survival of various components during storage or during regeneration, and the effect of sample size. In the case of genetic shifts, it is absolutely imperative that genebank managers be aware of this and take the necessary precautions to adequately represent the diversity within and accession during regeneration. Base collections should be preserved at sub-zero temperatures and at periodic (every 3 cycles) intervals be used for regeneration of working collections.

Loss of enzyme activity in aged seeds has been studied by many researchers and is the basis for one of the most commonly used seed viability and vigor tests, the topographical tetrazolium test, which measures non-specific dehydrogenase activity. The problem with most enzyme assays is that, although good statistical correlations with seed viability may be obtained, they do not reveal the mechanism for deterioration, nor do they discriminate between the cause and effect of deterioration. The TZ test itself is very subjective and relies on the ability of the analyst to interpret the degree and pattern of staining of a tissue as it relates to seed viability.

Studies on the enzyme cytochrome *c* oxidase (CcO) may offer a new approach to the study of seed deterioration. The catalytic function of this key bioenergetic enzyme is the reduction of molecular oxygen to water, the terminal step in the mitochondrial electron transport chain of all organisms. During seed germination mitochondria and CcO activity increase dramatically, and germination cannot occur in the absence of this key enzyme. We have examined the results of various inhibitor molecules, such as carbon monoxide, nitrous oxide and deuterium oxide, on the activity of CcO and their effects on seed germination and growth of roots

of beans (Sowa et al., 1993). Perturbing the active site of the enzyme affects seedling respiration, CcO activity and growth of the root. This has led us to propose that seed aging might be caused by a decrease in the efficiency of the enzyme. Partial denaturation of the enzyme could result in release of partially reduced oxygen species. These oxyradicals might be a source of lipid peroxidation and membrane alteration (Sowa et al., 1993), which has also been advanced as a prominent theory of seed deterioration.

Recent work has shown that membrane lipids undergo reversible phase transitions between the gel and liquid crystalline configurations, depending upon the moisture content (mc); this can be controlled by temperature. These configurations are important in determining the degree of permeability for the membrane. Alterations to the lipid moiety of the membrane, via peroxidation could lead to the production of free organic acids, an increase in free fatty acids, a decrease in unsaturated fatty acids, and a general production of toxic compounds. All of these can result in overall membrane dysfunction and deterioration of other macromolecules (proteins and DNA). Once again, this theory of seed aging as a result of membrane deterioration has led to a common viability/vigor test, the seed leachate conductivity test. Initially, this test consisted of soaking 50 or 100 seeds in distilled water and measuring the conductivity of the water after 24 hours (Matthews and Bradnock, 1967; 1968). The development of an instrument to measure 100 individual seed leachates has enabled us to look at a population of seeds. Forming the data into a frequency distribution can allow us to analyze the shape of the distribution, and how this might change as seeds deteriorate (Moore et al., 1988).

NEW PRESERVATION TECHNIQUES

New seed preservation techniques have concentrated on two factors, the temperature of storage and the optimum seed moisture content for storage. Recent research has examined the use of cryopreservation, and more recently, the lowering of seed moisture content to very low levels. Am important new approach has been taken at NSSL to develop a theoretical basis for seed storage protocols.

Cryopreservation: Cryopreservation refers to preservation at cryogenic temperatures. Cryogenic, is defined, although somewhat vaguely, as "of or relating to the production of very low temperatures." One view holds cryogenic refers to subfreezing (<0 ℃) temperatures; however, water may not freeze at this temperature and can supercool to as low as -40 ℃ ,

which is the homogeneous nucleation temperature of water. In this report cryopreservation will be restricted to storage in or over liquid nitrogen (LN, -196°C).

Survival of seeds exposed to temperatures at or near that of LN has been known for nearly 100 years. In experiments of Brown and Escombe (1897), seeds of 12 species, air dried to a mc of 10 to 12%, were slowly cooled in thin glass tubes, immersed in liquid air (-183 to -192°C) and held for 110 hours. After seeds were slowly warmed (over a 50-hour period) there was no apparent effect on germination. Research conducted at the NSSL has emphasized development of seed cryopreservation technology. Questions which are important in assessing this technology include: (a) Can seeds of a given plant species withstand being subjected to LN without loss of viability? (b) Does seed mc at the time of exposure to LN affect seed response? (c) How long will seeds remain viable in LN? (d) How do costs of LN storage compare with costs of conventional refrigerated storage? (Stanwood and Bass, 1987). To these questions the following may be added: (e) Can all orthodox seeds be cryopreserved? (f) Can recalcitrant seeds be cryopreserved? (g) Does cryopreservation have any genetic effects on the seed? Some of these questions have been answered or partially answered, but others may never be answered.

A large number of orthodox seed species have been shown to survive LN exposure (Stanwood and Roos, 1979; Stanwood, 1980; Stanwood and Bass, 1981; Stanwood, 1985). Physical damage (splitting, seed coat cracking) to seeds of some species (garden beans, flax, radish, soybean and alfalfa) has been reported (Stanwood, 1985); however, careful control of cooling rate, seed mc and rewarming rate may overcome this damage. For example, bean seeds exposed only to LN vapor (ca. -150°C) showed no breakage. One of the most important factors controlling seed response to LN exposure is mc. Fortunately, for most orthodox seeds, this factor is easily controlled.

One of the most commonly asked questions concerning the adoption of cryopreservation technology for seed germplasm involves the economics of LN vs conventional storage. Stanwood and Bass (1981) attempted to analyze costs for storing onion seeds in LN vs -18°C by making certain assumptions regarding equipment and facilities, yearly operations, and seed regeneration costs. Over a 100 year period, annual costs for maintaining one accession were $1.65 and $0.42 for conventional (-18°C) vs LN storage, respectively. Thus, yearly operational costs (mainly viability monitoring) and seed regeneration costs (resulting from seed deterioration)

were much less under LN storage.

In addition to seeds, many other forms of germplasm, including pollen, dormant buds, and apical meristems are amenable to cryopreservation. Indeed, cryopreservation may be most valuable for these forms and for storage of recalcitrant seeds, for which there is presently essentially no other alternative for long-term preservation.

Theoretical Basis for Seed Storage Protocols: Genebank managers desire to know the optimum conditions for storage to maintain seed germplasm for the future. It has been known for many years that dry, cold conditions will extend longevity: however, the optimum conditions and the theoretical basis for this are poorly understood because research on long-term storage necessarily takes many years to complete. Scientists have relied on experiments done under less optimal conditions and then extrapolated their results to predict longevity under optimal conditions via mathematical equations (Roberts, 1973; Ellis and Roberts, 1980, 1981). The assumption of these equations has been that the effect of water content on the rate of seed deterioration is a logarithmic relationship and thus, the drier the tissue is, the longer it will maintain viability (Ellis and Roberts, 1980; Ellis et al., 1986; Ellis, 1988).

One way to circumvent the problems that arise from making such assumptions is to analyze the physical state of water in seeds. Such analyses indicate, on a theoretical basis, the rates of deteriorative reactions in seeds with different mc and at different temperatures. Both of these parameters affect the state of water in seeds. It is relatively easy to demonstrate that the state of water binding in seeds is dependent upon temperature (Vertucci and Leopold, 1987; Vertucci, 1990). Recently we have also demonstrated that at very low seed moisture contents the seed deterioration rate begins to increase (Fig. 3) and, thus, there is an optimum moisture content for storage (Vertucci and Roos, 1990). This has recently been addressed from a thermodynamic perspective and it is now possible to predict the optimum moisture level as a function of the temperature of drying and the eventual storage temperature (Vertucci and Roos, 1993). This has important implications for genebanks desiring very long storage for base collections, of for storage of active collections at less than optimum temperatures.

The recent suggestion of using "ultradry" storage in areas where refrigeration is not reliable (Cheng et al., 1990; FAO/IBPGR, 1993; IBPGR, 1992) is based on substituting the benefit of low temperature for that obtained by reducing moisture content further. The problem is that using

moisture content per seed as the desired parameter is somewhat meaningless because it does not indicate the activity of water (the critical parameter in deteriorative reactions). RH in the air with which the seeds are equilibrated more accurately reflects water activity. Since seeds equilibrate to different moisture contents, depending upon their chemical composition, RH might be better used to specify optimal conditions for storage (Vertucci and Roos, 1990). The problem becomes then to define what is the optimum RH and what is the effect of temperature on this value. The most significant finding of this work is that the optimum seed moisture content for a given species increases as storage temperature decreases (Fig. 4).

OTHER TECHNOLOGIES

Before concluding this presentation I would like to briefly mention a few other technologies that are emerging and should have important implications for genebank managers. These include the dramatic increase in computer imaging techniques, non-destructive testing of seed viability, the potential for rescue of deteriorated seeds, and finally the storage of high moisture tissues, such as recalcitrant seeds, dormant buds, shoot tip meristems and pollen.

Video Imaging Technologies: High speed computers are an integral part of any genebank program as they contain all the data about accessions in a form that is readily retrieved. The advent of increased data storage capacity and the software to analyze video images has opened up the prospect of storing data in an entirely new format, namely a combined picture (image) plus the usual data associated with an accession. The analysis of the image can provide such data as seedling growth rate (Howarth and Stanwood, 1993) and seed/plant characteristics (Howarth and Stanwood, 1992). Combining such data about a seed accession on a CD ROM can give the user (breeder) an objective assessment of the germplasm of interst and should assist in the collection and evaluation of new data about the genetic variability within a species.

Non-destructive Testing for Seed Viability: We are currently investigating several approaches to determine seed viability non-destructively. These include: use of seed X-ray analysis; calorimetric analyses; examining leachate conductivity of partially imbibed seeds, followed by drying back to the original mc; using infra-red (FTIR) analysis of minimally imbibed seed. Of these, the FTIR technique appears to be the

most promising and has been used to assess viability of cell suspensions and pollen (Sowa and Towill, 1991; Sowa et al., 1991), and bean seeds (*Phaseolus vulgaris*) (Sowa and Roos, 1992).

Rescue of Deteriorated Seed: Unfortunately, genebanks occasionally receive seed in very poor condition, with only minimal germination. In many cases this represents valuable germplasm in danger of being lost. Research is underway to rescue this material. The approach usually taken is to provide optimum conditions for germination, including control of microorganism growth, seed priming, and providing various nutrients and/or hormones. Alternatively, embryos may be excised and grown in culture to produce plants which can then be transferred to the greenhouse of field, as appropriate. Regeneration of plants from callus and protoplasts is also a possibility and new reports on special techniques appear almost daily in the literature.

Other Germplasm Preservation: Although this presentation has mainly concerned preservation of orthodox seeds, it should be recognized that important breakthroughs have recently been made on the storage of recalcitrant seeds (Vertucci et al., 1991; Wesley-Smith et al., 1992). It should soon be possible to store these materials, probably in liquid nitrogen, on a routine basis. Similarly, long-term storage of non-seed germplasm, particularly of clonally propagated species, is now possible (Towill, 1989; 1990; Towill and Jarret, 1992).

CONCLUSION

Germplasm preservation is fundamental to future crop improvement. The new information now emerging should answer many questions about seed longevity, deterioration, and preservation. Modern genebanks, such as the one we are dedicating this week, can not limit themselves to orthodox seed storage. We must be capable of preserving germplasm in several forms. The facility here in Taiwan should now take its place in the world community to preserve germplasm for the benefit of all Humankind. Finally, I would like to once more thank the organizers of this symposium for extending an invitation to me to participate in this joyous event.

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Table 1. Seed longevities of 100 or more year. (data from Roos, 1989)

	Species	Longevity	Comments	Reference
1.	*Lupinus arcticus* S. Wats	10,000 +	^{14}C dating of assoc. rodent bones	Porsild et al., 1967
2.	'Mummy' wheat and barley	2000-3000 +	Egyptian tombs	Barton, 1961; Bewley & Black, 1982; Justice & Bass, 1978
3.	*Nelumbo nucifera* Gaertn.	3075	^{14}C dating of assoc. wood canoe	Libby, 1954; Wester, 1973
4.	*Chenopodium album* L.	1700	Archeological dated soil samples	Odum, 1965
	Spergula arvenis L.	1700		
5.	*Nelumbo nucifera*	120-1080	^{14}C dating, geologic, and historical records	Godwin & Willis, 1964; Libby, 1951; Ohga, 1923; Ohga, 1927
6.	Various weed species (36 different species)	110-600 +	Archeological dated soil samples	Odum, 1965
7.	*Canna compacta* Rosc.	530-620	^{14}C dating of assoc. materials	Lerman & Cigliano, 1971; Sivori et al., 1968
8.	*Nelumbo nucifera*	237	Museum specimen	Anonymous, 1942
9.	*Medicago polymorpha* L.	200	From adobe bricks	Spira & Wagner, 1983
	Hordeum leporinum Link	200	Positive TZ reaction	
	Malva parviflora L.	183-200	Positive TZ reaction	
	Trifolium sp.	193	Positive TZ reaction	
	Chenopodium murale L.	183	Positive TZ reaction	
	Chenopodium album	143	Positive TZ reaction	

Table 1. (Continued). Seed longevities of 100 or more year. (data from Roos, 1989)

	Species	Longevity	Comments	Reference
10.	*Cassia multijuga* A. Rich.	158	Museum specimen	Becquerel, 1934
11.	*Albizzia julibrissin* Durazz.	147	Museum specimen	Anonymous, 1942
12.	Barley and oats	123	Building cornerstone	Aufhammer & Simon, 1957
13.	*Hyoscyamus niger* L.	116	Museum specimen	Odum, 1965
14.	*Cassia bicapsularis* L.	115	Museum specimen	Becquerel, 1934
15.	*Goodia lotifolia* Salisb.	105	Museum specimen	Ewart, 1908
	Hovea linearis (Sm.) R. Br.	105	Museum specimen	
16.	*Malva rotundifolia*	100	Buried seed	Kivilaan & Bandurski, 1981
	Verbascum blattaria L.	100	Buried seed	
	Verbascum thapsus L.	100	Buried seed	
17.	*Trifolium pratense* L.	100	Museum specimen	Youngman, 1951
	Lotus uliginosus (L. *pedunculatus* Cav.)	100	Museum specimen	

Table 2. Longevity of 15 vegetable seed species. (Roos and Davidson, 1992)

Species (common name)	Age (yr)	Germ. (%)
Abelmoschus esculentus (okra)	50	34
Allium cepa (onion)	52	20
Beta vulgaris (beet)	59	8
Beta vulgaris (Swiss chard)	48	70
Capsicum annuum (pepper)	51	28
Citrulus lanatus (watermelon)	58	32
Cucumis melo (muskmelon)	58	42
Cucumis sativus (cucumber)	58	28
Daucus carota (carrot)	46	24
Lycopersicon esculentum (tomato)	60	82
Phaseolus vulgaris (bean)	48	24
Pisum sativum (pea)	51	94
Solanum melongena	56	4
Spinacia oleracea (spinach)	45	60
Zea mays (corn)	50	56

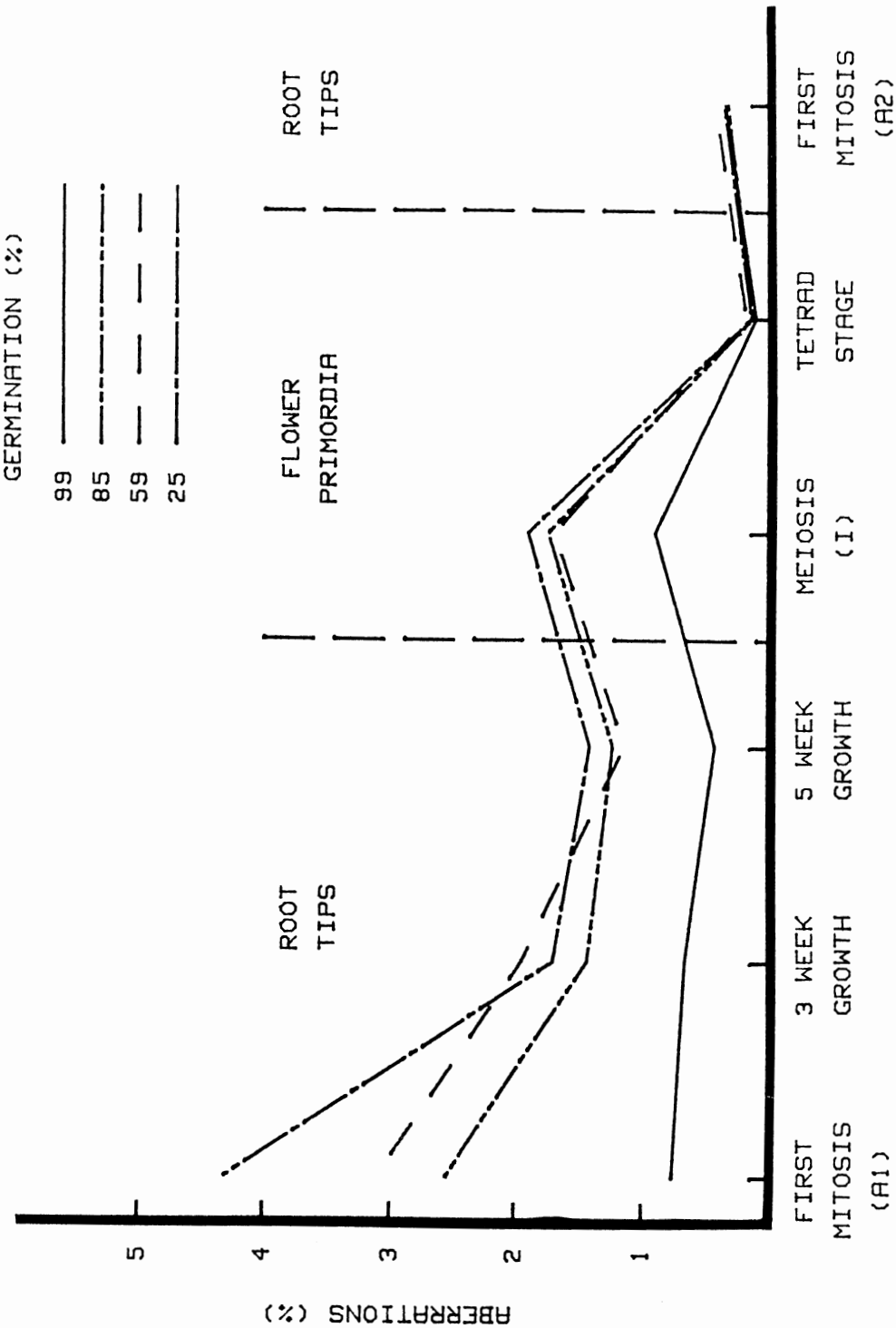


Figure 1. Effects of plant growth on elimination of aging-induced chromosomal aberrations in barley seeds. (data from Roos, 1982)

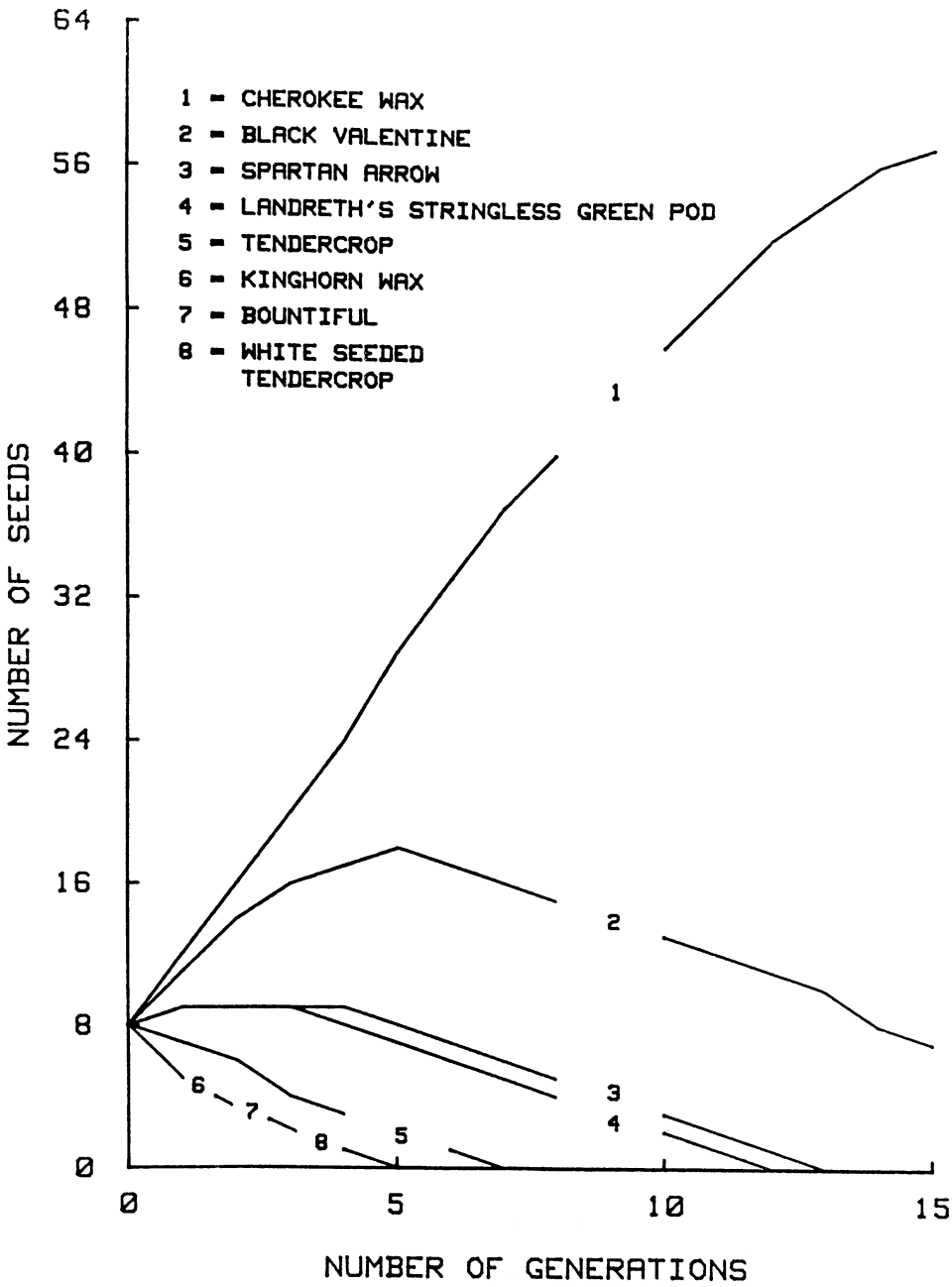


Figure 2. Computer simulation of genetic shift in composition of a mixed snap bean population following several generations of seed aging (to 50% population germination) and seed increase. The initial population consisted of 8 seeds of 8 cultivars (population size = 64 seeds). After 15 generations only two cultivars remain in the population. (data from Roos, 1984b)

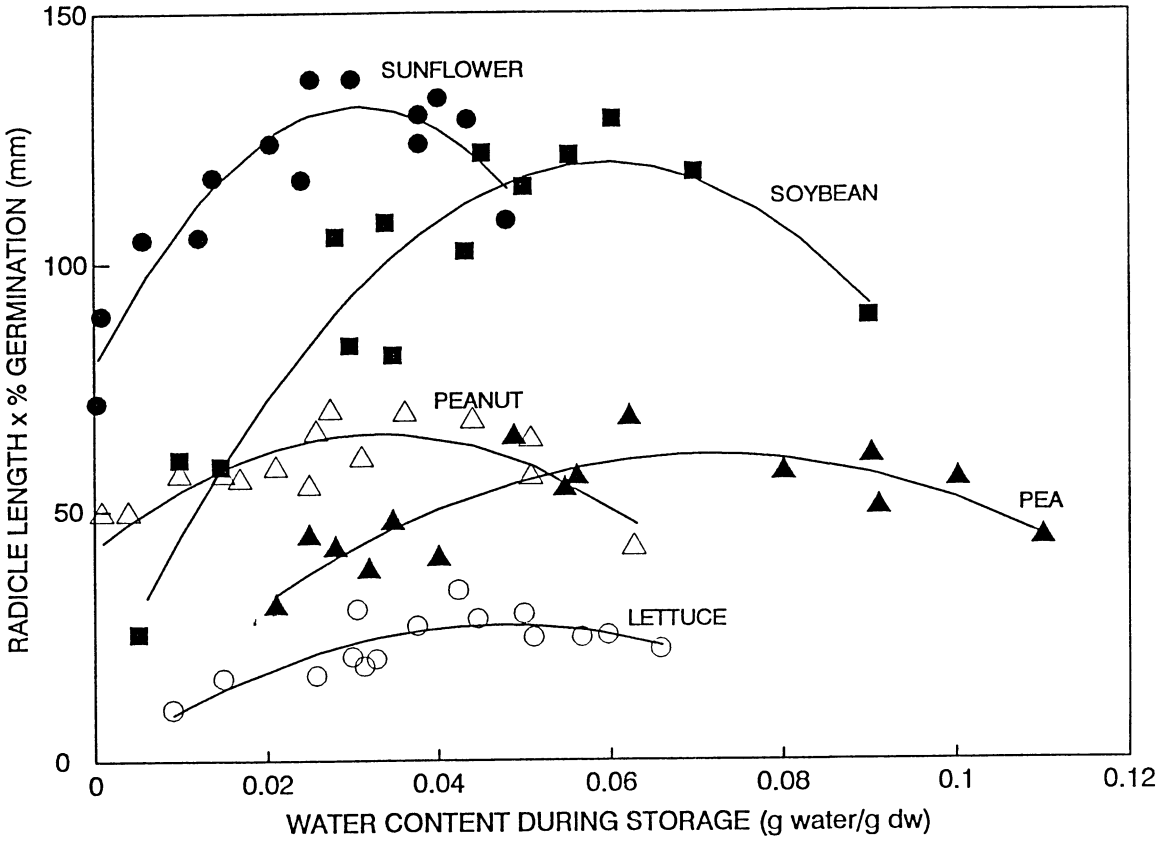


Figure 3. Efect of moisture content during storage at 35℃ on seed vigor. Data are presented for soybean and sunflower stored for 2 months and peanut, pea, and lettuce stored for 4 months. Vigor is expressed as the germination index: the mean radicle length × the percentage germination. The curves are drawn from fitted second order polynomial equations. (data from Vertucci and Roos, 1990)

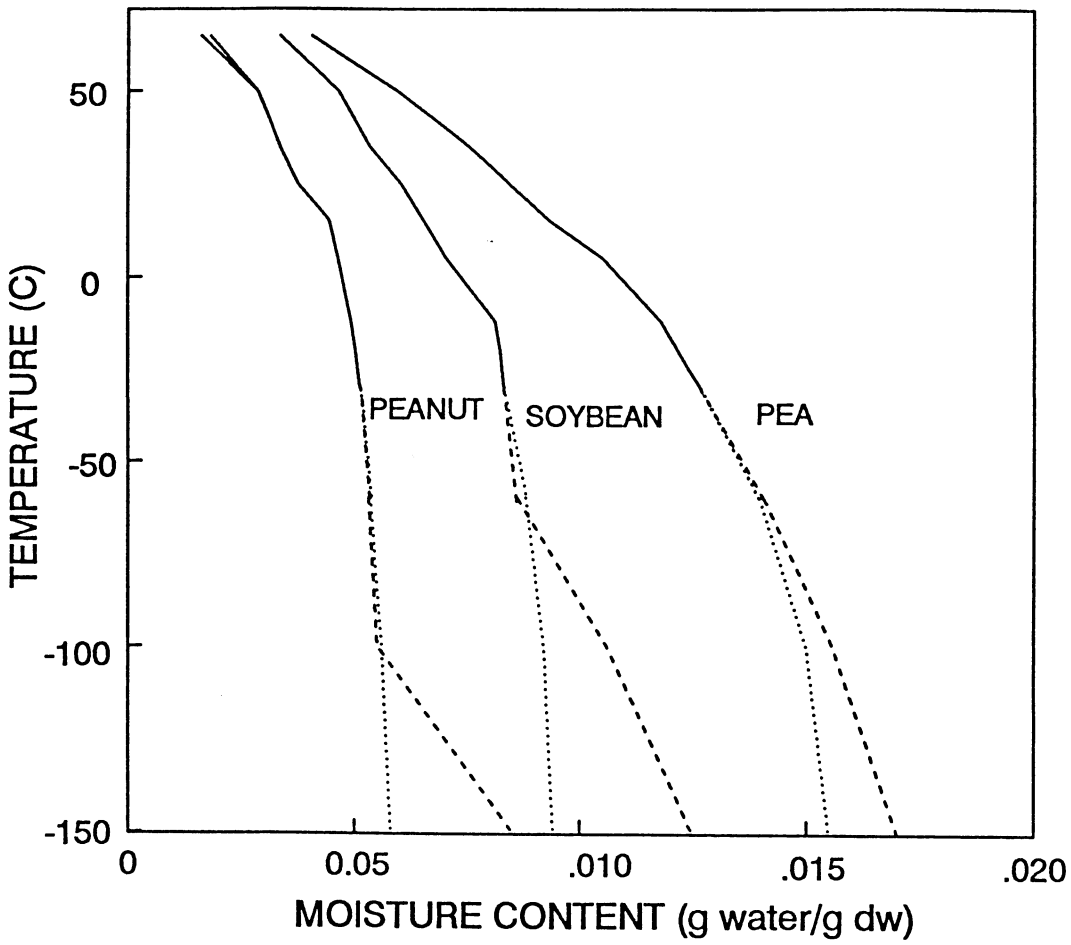


Figure 4. The optimum moisture content for storage of pea, soybean and peanut seeds at storage temperatures between-150 ℃ and 65 ℃. Dashed and dotted lines represent determinations based on isotherms calculated from heat capacity measurements and van't Hoff analyses, respectively. (data from Vertucci and Roos, 1993)

Plant Germplasm Conservation: Perspectives for the 2000s. 83-99 (1994). Taiwan Agr. Res. Institute

CURRENT AND FUTURE DEVELOPMENTS IN CLONAL GERMPLASM CONSERVATION OF VEGETATIVELY PROPAGATED CROPS

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INTRODUCTION

Plant genetic resources are rapidly erod or vanishing due to deprivation of habitats and selective reduction, leading to genetic vulnerability endangering the plant industry and future needs (Frankel and Soule 1981). The conservation of germplasm becomes an urgent necessity, asit plays a crucial role in the improvement of crop variety and food production. The seeds or pollens could serve as functional vector carrying or transmitting genetic information and readily preserved as representive of species genome or genetic diversity. But majority of perennial crops such as fruits, nuts, some tuberous crops and ornamental trees are propagated vegetatively. Ten of these crops like potato, sweet potato, cassava, grape, sugarcane, orange, banana, apple, yam and mango were ranked within the world's top 30 crops (Ford-Lloyd 1990). Asexually propagated crops are characterized by long juvenility and highly genetic heterozygosity, which are not easily duplicated by seeds. Most of tropical and subtropical fruits, large seeded trees and economical importment crops as palm, cocoa and rubber are considered as recalcitrant seeds which are sensitive to dessiccation and chilling (Hartmann et al, 1991), the short longevity becomes unsuitable for long-term seed storage. Additionally, the sexual infertility exists in triploid bananas--its genetic variability mainly derived from somatic mutation, and the double-flowers crops and some breeding lines are difficult to produce seeds. The germplasm maintenance of sterile crops are dependent on vegetative propagules.

The strategy of germplasm conservation depends on the nature of the material and on the objective and scope of the activity (Ford-Lloyd

and Jackson 1986; Towill 1988). For practical purposes in production of uniform clones and due to the limitation of seed problem, the asexually propagated crops are used to place vegetative parts or plant form in a repository maintained *in situ* and *ex situ*. Their contributions and disadventages would be discussed. Basic efforts on long-term conservation of genetic resource are to maximize the diversity and prevent genetic erosion, to minimize maintenance cost and to enhance utilization efficiency. In reality, the conservation of each crop in a clonal genebank is much expensive and less efficiency than in a seed genebank. Considering economy of scale and efficiency, alternative method of *in vitro* conservation of tissues or DNA have been developed. The application of tissue culture for clonal germplasm conservation of vegetatively propagated crops is now promising. The achievement of recombinant DNA biotechnology will be helpful to maintain the genetic diversity through *in vitro* DNA conservation. The benefit and limitation of *in vitro* conservation of plant tissue and genome will also be discussed.

In Situ Conservation

Maintenance of wild species or crop relatives should be in original habitats. The planned area serves as flora homeland letting boundary any set of plant community to act as an unit under influence of ecosystem and protection from human destructive impact. The advantage of *in situ* conservation benefits the population open to gene flow undergoing genetic recombination, gene exchange and mutation accumulation under ecogeographic interaction and competition influence. This permanent habitat preservation was considered as dynamic and evolutinary conservation (Frankel and Soule 1981). Even the incidence of natural selection force and evolutionary changes was subjected to slow processing but critical for creating novel genetic variability particular regarding coadaptation characters.

In order to enhance efficiency on dynamic change and future needs of genetic resources, some man-made efforts such as priority setting, selection design, population analysis, mangement and monitoring are required to be manipulated (Vaughan and Chang 1991). Vavilov indicated that the nativeland of a particular crop usually has great genetic diversity. Additional four plentiful diversity zones were added to the original Vavilov's eight centers throughout the world (Smith 1969). For global crop genetic resources, the diverse center is the best candidate for site

setting. Actual size needed for individual conservation was suggested to be hundred ha maintain thousand breeding individuals in 40 ha and extra 60 ha for natural regenerating stand (Kemp et al., 1970).

However, the woody plants, fruit trees, ornamentals and fuel trees were not included in the Vavilov centers. Recently, the information of genetic diversity and germplasm conservation in temperate fruits and nuts was well-organized by IBPGR and ISHS (Moore and Ballington, 1992). Most of related wild species and its population are rare and have scattered distribution at marginal land or mountainous area that can be assigned as nature reserve or national park to provide the last stand for survival. Tropical primary rainforest land is always rich in fruit germplasm and potential use. An investory was made of 50 ha site at Pason nature reserve in Peninsular Malaysia in which ca. 340,000 tree flora out of a total 820 species composing 76 edible fruits, which included 12 *Mangifera,* 13 *Garcinia,* 10 *Artocarpus* and 5 *Nephelium* species (Saw et al., 1991). Most of the wild species are not characterized yet, but the potential value as genetic resources is very large. Some of the species density is lower than 1 adult tree per ha behind the conservation threshold. How the dynamic change in population density, long term stability and species-specific factor or breeding behave are worth investigating. One unique geographic area, Cape of Goodhope, is occupied by fynbos flora with delicatedly shaped or attenuated leaves. The Cape area was successfully reclaimed to a nature reserve with 7750 ha maintaining 1100 species, composed of three major elements as 39 Ericoid, 29 proteoids and 52 orchids (Fig.1). This colorful ornamental habitat genebank provides a nature class and entertains people. Many of the ornamental species were bred and released to the market.

The specific wild species related to local or national crop industry are usually emphasized in priority conservation. Wild species or primitive species always possess abundant variability and include desirable characters useful for crop improvement (Hyland, 1970; Shii, 1983). Many under-exploited wild species potentially develop into new crops (Possingham, 1990). There are progressing in breeding techniques, such as overcoming sexual barrier and asexual transfer of aline gene into plants (Fig. 4). The germplasm enhancemant or direct utilization of distant species would become much feasible. Regardless of potential, the rescue and recovery of rare and endangered species are subjected to national responsibility. In Taiwan, seven nature reserves were respectively established for rare species: *Cycas formosana, Pleione taiwaniana, Kandelia*

mangrove, Keteleeria davidiana, Amentotaxus formosana and one hydrophte *Isoeles taiwanensis,* each is bounded in 50 to 100 ha protected zone (Simpson et al., 1992). The endemic species as *Malus formosana, Pyrus koehnei, Phalenopsis amible,* and some of native *Vaccinum, Actinidia, Rhododendron, Rosa* species are also in danger situation required a close protection and recovery, Westwood (1977) indicated that the *Pyrus koehnei* is resistant to fireblight, leaf spot, pear root aphid, waterlogged soil and low chilling requirement. *In situ* conservation performs as natural germplasm reactor of wild species. The positive feedback would be fruitfully.

Ex situ Conservation

The collected germplasm of perennial species departed from their original habitat is being maintained in botanic garden, experiment station, research institute and by commercial enterprises, nursery or homegarden. Historically, the botanic garden and aboreta play important role in collection and identification of wild species (Frankel and Soule, 1981), which also represent the oldest form of *ex situ* conservation in Europe back to the 15th and 16th centuries. Now, there are close to 700 botanic gardens of varying size in the world. From the 17th century in Florence Italian, a series of fruit paintings described various patterns and colors in citrus, cherry, grape, fig and others They recorded more than 30 cultivars in each crop (Fig. 2), reflecting the local people pay attention to the conservation and utilization of fruit germplasm. The Royal Kew Botanic Garden was initially established in 1759 with 5 ha, and expanded to the present 121 ha preserving more than 30,000 plant types. The botanic gardens also have made remarkable contribution to the exploration and conservation of tropical flora, and raised the development of greenhouse conservatory on modification of microclimate condition for protection of the introduced tender plants. The model of *ex situ* conservation with plant community was also simulated in some botanic gardens. The botanic gardens also actively taken part in conservation and recovery of rare and endangered species (Druy, 1974), some of the endangered species were and successfully introduced into horticultural trade.

For agricultural purpose, all the desirable characteristics related to yield, quality or resistance are worthy of exploration, and preservation in a field genebank. Herbaceous clonal lines are usually easier to maintain as seed population than the woody lines, except passion fruits or grapes

are easily duplicated and preserved by seed. Most of fruit trees, the collection of related wild and primitive species or variety might use the seeds as propagule. The germplasm of domesticates, landraces and cultivars are preferred to adopt vegetative part as preservation materials, which are propagated with selfed-roots, or grafted on the dwarf rootstock for saving space. The clonal repository serves as basic and active conservation site, ready for evaluation and utilization, and provides breeding materials. This clonal genebank is under influence of agroecosystem, we have to aware the possible high risk of field genetic erosion, and needed to protect from suffering of natural disaster and pathogen infection. The spectrum of genetic integrity is also much restricted than seed genebank. Large amounts of space and labor are required to maintain the small plant populations.

It is not feasible to preserve all the crop clonal germplasm at one repository as seed genebank. The priority is focusing on the national plant industry. The conservation network of regional repositories of fruits and nuts was well-planned in U.S.A. The NRPGR is the first of 12 proposal clonal genebanks opened in 1981 at Corvallis. It is mainly in charge of evaluation, maintenance and distribution of the national collection of pear, hazelnut, hop, mint and small fruits(Anonyous, 1981). The other national clonal germplasm repositories take part in conservation of regional major fruits were completed by 1988 (Fitzerald, 1988). The collected clonal germplasm are preserved in the field, greenhouse and other methods. The IBPGR was organized in 1974 for planning the global germplasm network and international collaboration program on basis of crop-by-crop. Comparatively, the germplasm of tuberous crops and its clonal genebanks are relatively well-established. The collected accession among potato, cassava, sweet potato and yam are recorded as 42,000, 8,000 and 10,000 respectively (Plucknett, 1987). It is a heavy load in dealing with these amounts of collected germplasm by clonal preservation. The primary genebank of potato is maintained at Huancayo in Central Peru at 3300m altitude, reduced the living accesions to 5,000 accessions, and the complementary centers are also established at the other two or three countries. Cassava genebank is established at CIAT, maintaining 4,677 accessions in 1991. One active collection of about 1,219 sweet potato accessions was preserved at AVRDC, usually regenerates twice a year with cutting (Tay et al 1987). It is estimated that the collected accessions in potato was almost covering the existing genetic integrity. But the germplasm of cassava is only included 35 % landraces and 5 % wild

species pooled in genebank (Ford-Lloyd, 1990).

The status of germplasm aquisition of fruit trees especially the tropical fruits were much limited, and varied from crop to crop. For instance, the cashew and avocado respectively have 3,600 and 2,234 accessions in field genebanks, but the *Diospyros* only have 129 accessions, the coverage percentage of landraces and wild species are not clear yet. The papaya, guava and Brazilian nut are ascribed to neglected germplasm. Basic collection of bananas are undertaken in Southeast Asia country, a total of 323 accessions are preserved at Kasetsant University of Thailand (Sylayoi and Chomchalow 1987). The banana collections at other countries including Malaysia, Vietnam, mainland China and Papua New Guinea ranged from 200 to 100 accessions. The international organization of banana and plantain germpeasm set a transit center of *Musa* germplasm at a neutral country, Belgium, routinly preserved one thousand accessions *in vitro*.

In order to ensure wide spectrum of germplasm conservation and reduce redundant accessions. Except morphological diagnosis, it is necessary to develop other genetic markers for accurate characterization of the clones (Peacock, 1984). For example, the diversification and origin between genomic groups and subgroups of edible triploid banana germplasm showed to be feasibly differentiated by biochemical markers such as isozyme *Got* genefamily as showing in Table 1, suggested that *Got* 2 gene could serve as genome marker. Both allotriploid AAB and ABB genomic groups easily differentiated due to allelic dosage effect. The Got3 could perform as subgroup marker, showed that autotriploid AAA subgroup Red, Gros Michel and Cavendish are of different genetic origin. Abundant variability in subgroup Cavendish might be derived from somatic mutation. The identification of clonal variation within subgroup required other molecular markers. It was demonstrated that DNA fingerprinting techniques could serve as a powerful tool in surveying phylogeny and discrimination of cultivars (Jarret et al., 1992, Kraemmer et al., 1992). As listing in Figure 3. The diverification of *passiflora* species could be illustrated by the RFLP of cp DNA. and evided that yellow passion fruit is differentiated from purple passion fruit (Do et al., 1992).

In vitro Conservation

The plant organ, tissue and cell are inducible to regenerate

quantatively morphological bipolarity individuals under *in vitro* conditions. The pathway of *in vitro* regeneration could be achieved following shoot-tip microcutting, axillary bud proliferation, adventitious bud formation and somatic embryogenesis, depending on explant attributes and culture medium. Both organogenesis and somatic embryogenesis are based on serial cell duplication and differentiation. All the regenerants come from cloning are identical to the mother stock. Therefore, small pieces of explant could represent the germplasm used as clonal storage material. Morel (1975) suggested that meristem culture technique can apply to the long-term storage of cultivated plants. Dale (1976) calculated that 800 cultivars of grape could be stored *in vitro* in a few square meters of space instead of one hectar field genebank. The controlled *in vitro* storage could prevent the erosion as fields suffer from natural catastrophe or disease infection. It is also helpful in maintaining and multipling specific genotypes such as sexual infertility, male sterility and self-incompatible or haploid lines. The test tube plantlets serve as safe bridge for transport and international germplasm exchange.

There are three major aspects when considering tissue culture for genetic conservation: 1. Genetic stability should be maintained. The somaclonal variation might be encountered due to perexisting and induced mutation or activated transposition (Scrowcroft and Larkin, 1982). Choosing shoot-tip as storage material, utilizing multiple shoot proliferation, will reduce the subculture generations and given minimal medium, all these manipulations are helpful to secure the stability. The testing of genetic purity is necessary for ensuring trueness-to-type of each accession in reliable maintenance. 2. Absence of disease must be guaranted, the target accession should pass through through healthy diagnosis. The initial eradication could be obtained by apical meristem culture or micrografting method. During storage period, air carrying infection such as mite and mold might destroy the accessions. Giving filtered aeration, low humidity and cold room temperature are useful. 3. The regenerability retention, the regeneration pathway and capability might vary with cultivars, tissue and medium. Some woody plants are difficult to regenerate. We have to overcome the problem through *in vivo* or *in vitro* rejuvenation treatment (Pierick, 1990; Hammatt and Grant, 1993), or choose high organogenic or embryogenic tissue as preserving materials. The storage period is actually based on the explant and plantlet longevity of regeneration potential *in vitro.*

In vitro conservation could be optionally conducted with slow

growth and cryogenic system for working collection and basic collection respectively (Roca et al., 1983). The slow growth method is to culture the shoot tip or bud under modifying condition. Let the plant alive but retarded growth processes through physical or chemical restriction. Longer storage can be subcultured at intervals. This storage method has considerable advantage in that the stored material is readily available for use, can be easily visible to be alive, and accession is rapidly to be duplicated when it is required. Various efforts on nutrient, osmotic stress, gas component, carbon sources, retardants and environmental factors have been imposed to restrict the growth (Bajaj, 1979; Withers, 1980). An alternation of the physical conditions most often by a reduction of the temperature, the cassava, sweet potato, coffee and lilies, given 15-20 ℃ temperature with minimal medium, one stored growth cycle could be extended to 12-18 months. The potato, strawberry, pear and apples can be stored at 0-6 ℃ as long as 2-3 years for one generation. Sometimes inhibitors such as CCC, ABA are used. Mix (1984) used nodal explants of 220 potato cultivars as starting material for long-term storage. Plantlets were maintained at 10 ℃ in the presence of N-dimethyl-aminosuccinamid acid. The adventitious buds of banana formed at MS medium containing higher agar concentration developed into corm-like structure, which were close to dormant state for one year still remaining alive, and rapidly sprout when decreased agar concentration or in liquid medium (Ma and Shii, 1974).

Slow growth storage of germplasm become a routine technique in conservation of most tuberous crops. Over 1300 cassava accession at CIAT germplasm center have been transformed into *in vitro* conservation. 6000 clonal accessions can be accomodaled in 5x6x3m storage room, using 5 vessels of 5x14 cm per accession (Roca et al., 1983). The INIBAP organized a transit center for *in vitro* storage and exchange of *Musa* germplasm in Leuven, Belgium. The shoot tip of each accession was multiplied and stored under 15 ℃ and 2,000 Lux lighting condition according procedures developed by Baneejee and Langhe (1985). For healthy diagnosis and field evaluation, parts of each clone were send to CIRAD (France), QDPI (Australia) and Philippines. As soon as the transit center receives negative indexing, each accession enters the medium term storage at a number of 20 cultures, a total of 1032 accession are preserved for research and germplasm exchange (DeSmet and Van den Houwen, 1991).

Cryopreservation is based on the reduction and subsequent arrest of metabolic function by the reduction in temperature of materials at ultra

-low temperature while maintaining viability. Bajaj (1977) showed the prospects of cryopreservation for the establishment of germplasm banks, which has numerous advantages. The propagule can be stored, with no need to subculture; retain morphogenetic potential and prevent aging; save space and manpower. Basic efforts of cryopreservation need to enhance the propagule tolerance in freezing process, and prevent the occurrence of ice crystallization and recrystallization during cooling and thawing, which do mechanical damage to the cells (Nitzache, 1983). Preacclimation, cryopotectants and prefreezing are amenable to minimize the incidence of intracellular ice formation, increase the stability of plasma membrane and decreased the lowering nucleation temperature at -42 ℃ . The nucleation temperature of nonacclimation is -15 ℃ (Steponkus, 1985).

The type of preserving materials such as shoot tip, apical meristem or embryos are considered the most desired propagules for germplasm conservation regarding genetic stability, but the survival percentage is often 60 % or lower (Kartha, 1985; Towill, 1988). Pregrowth cool acclimation or encapsulation-desiccation system have been applied to improve the cryogenic tolerance. The more cell homogeneity in the propagule, such apical meristem, as callus or cells, would have the higher viability under ultra-low temperature condition (Henshaw, 1984). But the more complex part is to regenerate after thawing and risk of somaclonal variation. Protopalsts of *Atropa, Nicotiana* and *Datura* subjected to -196 ℃ storage in presence of DMSO, mannitol and sucrose cryoprotectants, after 35 ℃ thawing, the former two obtained 71 to 53 % regeneration, even the somatic hybrid protoplasts still achieved 10-18 % survival (Bajaj, 1989). Predessiccation with vitrification solution containing ethylene glycol, PEG, DMSO and sorbitol that have been shown to enhance the cryogenic survival of various types of stored materials (Sakai et al., 1990; Towill, 1990; Langis and Steponkus, 1990).

The factors of pregrowth condition, cryoprotectant formulation, cooling rate and thawing temperature have greatly affected the survival, which are needed to be defined for different crops. Withers (1984) recommended the cryopreservation procedures as follows: for cell propagule, pregrow on standard medium or medium supplemented with 6 % mannitol for 4 to 7 days. Then harvest during active growth cryoprotected with 0.5M DMSO, 0.5M glycerol and 1M sucrose or proline. Dispense into ampoules freezon at 1 ℃ /min to -35 ℃ ; thereafter, hold at -35 ℃ for 40 min. then transfer to liquid nitrogen. These stored cells thaw rapidly in warm water at 40 ℃ and recover on semi-solid medium. The procedures

for protoplast are very similar to the above recommendation; or substitute the cryoprotectant with 5 % DMSO added with 10 % glucose.

DNA *in vitro* Conservation

Major plant genomes are localized in the nucleus, and two other small genomes exist in the chloroplast and mitochondria. Interms of DNA content, the nuclear genome size varies from species to species, such as soybean and wheat are respectively calculated as 8.69×10^8 b.p. and 1.5×10^{10} b.p. DNA, and close to 0.9 and 16.5 pg per haploid cell (Benett and Smith, 1976; Roger and Bendich, 1988). The genetic informations are loading in chromosomal DNA, although the DNA content not all means to be functional sequences but substantial constant with specific species. The genetic diversity can be elucidated by the degree of DNA homology. Therefore, the collected and reserved genomic DNA is capable to represent as genetic resources.

Plant DNA can be isolated and purified routinely (Murray and Thompson 1980, Watson and Thompson, 1988). The DNA yield changes with crop species and tissue types, the leaf samples usually have high yield average 14-49 μ g/mg tissue. It is only need 55mg of tissue to obtain 2.9 μ g DNA, that is enough for detecting the single copy gene (Roger and Benedich, 1988). Even a few copies of isolated DNA, that can be amplified rapidly through polymerase chain reaction *in vitro.* It was demonstrated that the mummified plant tissue is still extractable to obtain DNA (Roger and Benedich, 1985). It turned out, the freeze-drying tissues can be stored and serve as delaying DNA conservation, considered potentially to save endangered germplasm, and could be used whenever we needed (Adams, 1992). The purified DNA with nuclease-free is able to be preserved in alcohol solution indefinitely. Theoretically, the genetic resources of all the global flora DNA are feasible to be maintained in one house (Ford-Lloyd, 1990). The DNA library could be also achieveable by in vivo deposition and amplification in prokaryote cells.

Utilization of DNA genebank could be approached by genetic engineering. The isolated genes could be optionally delivered into target cells through vector, electroporation, particle bombardment or direct uptake and pollen-tube pathway. *In vitro* culture of the transformed cells regenerate into plantlets via organogenesis or embryogenesis, so called transgenic plants. A few of the desirable sequences related to resistance of disease, insect, herbicide, drug or cold stress, and the genes involved in

developmental regulation of storage proteins, pigmentation and fertility are already available, and successfully transformed and expressed in the transgenic plants (Fisk and Dandeker, 1993). The progress in biotechnology would be delightful for the use of conserved DNA germplasm, to improve the crop without limitation by phylogeny relationship and sexuality. The introduction of foreign genes do not need to change the genetic background of the breeding materials. This molecular breeding is highly potential for improvement of vegetatively propagated crops, especially to the sterile crops, such as bananas. The transformed cells developed into somatic embryos and positive expression is showing in Figure 4. (Liao et al., 1992).

The development of nake DNA bank for conservation of the genetic resources reveals as the most economical way. In consideration of molecular biology in utilization of conserved DNA, the progress in genomic sequencing and mapping is still very limited. Most of the genes involved in quantitative traits are not well-identified yet, and the engineered sizes of foreign genes restricted to a few copies. However, if the diverse genome or the DNA sequences could be preserved particularly for the extinction threatened or endangered species, it would serve to make more readily available and expanded the breadth of genetic resources whenever required, and to include those of the past (Ford-Lloyd, 1990).

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Fig. 1 *In situ* conservation of fynbos flora in nature reserve at Cape of Goodhope.



Fig. 2 Utilization of genetic diversity in fruit industry by ancient people depicted by paintings.

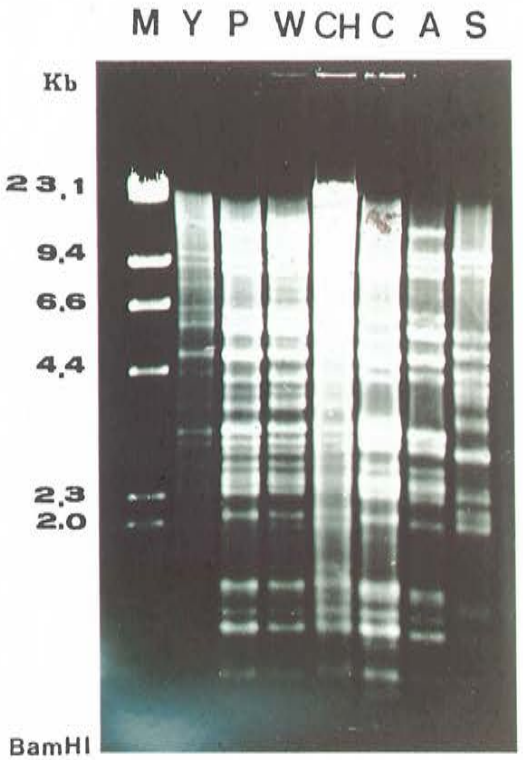


Fig. 3 Characterization of *Passiflora* species by cpDNA fingerprinting through Hind III digestion. Y: *P. edulis* f. *flavicarpa*, P: *P. edulis*. W: wild escape of *P. edulis*; CH: *P. coccinea* x Y; C: *P. alata*, S: *P. suberosa*.

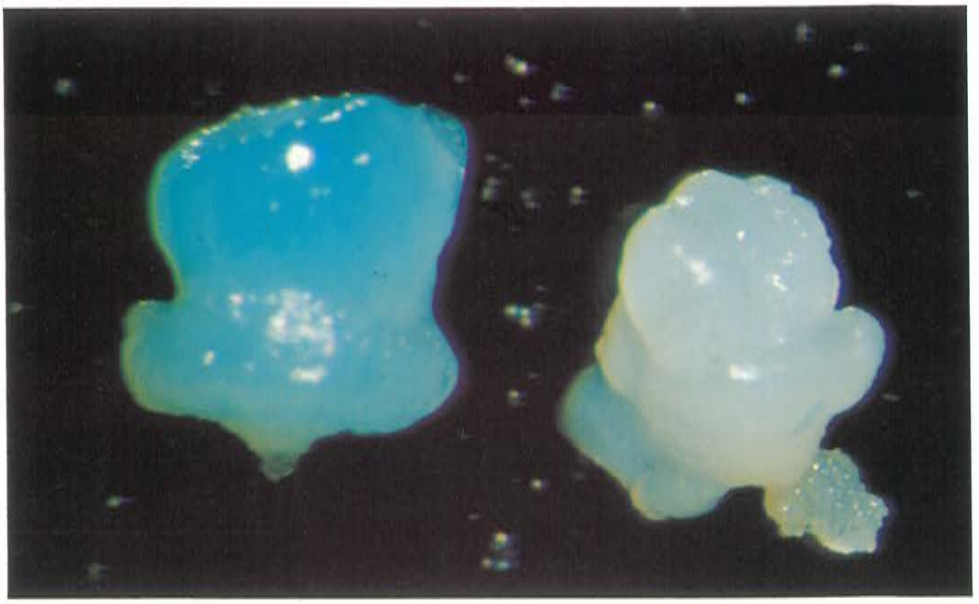


Fig. 4 The asexual trausfer of foreign DNA transformed into triploid banana culture cells and regenerated into transgenically somatic embryos (left one)

Table. 1 Characterization of germplasm diversity through Got genefamily markers on the introduced polyploid banana clones.

	genotypes and genomic groups			
Got 2 / Got 3	*FFF* AAA	*FFS* AAB	*FSS* ABB	*SSS* BBB
genotypes and subgroups	*aah* Red (5) *bbh* Gros Michel (7) *bhh* SH3436 (3) *bch* Cavendish (57) — — AAAA *FFFF* IC_2(4) Bodles altfort (1)	*bbh* Raja (8) *bch* Kanaba (5) *bhj* Kabok (15) *cch* 3-6(2) *dhh* 3-1(1) *hhh* Laknau(3) *hhj* Corne plantain	*bhh* K. hakmuk (2) *bjj* Apple (2) *hhh* P. bayu (4) *hhj* P. awak (5) *hjj*	*hhh* Malaca (2) — — ABV FSF_2 Tibok (1) — — BVX FF_2S_1 Pitogo (4)

Note: 1. In parenthesis represents the number of clones. The accessions of SH3436 and AAAA genomic group are man-bred clones.

2. The *Got2* genotypes of wild species *Musa acuminata* (AA) and *M. balbisiana* were assessed as *Got2-FF* and *Got2-SS* respectively.

3. All of the alternative *Got3* alleles present in these triploids are also detectable in wild species. *Got3-a, b, c, d* types are specific to A genome. *Got 3-h* is common in both of A and B genomes. *Got3-J* is specific to B genome.

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THE ROLE OF BIOTECHNOLOGY IN EXPLOITING AND USING PLANT GERMPLASM

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ABSTRACT

Following the inauguration of this National Plant Genetic Resources Centre, it is particularly timely to assess the role of biotechnology in relation to plant genetic resources conservation now and into the next century. Biotechnology needs access to plant germplasm to enable the desired novel plants and plant products to te produced; conversely plant genetic resources can often greatly benefit from the procedures used in biotechnology. Plant germplasm conservation is central to the strategies, particularly of Developing Countries, with regard to the acquisition and development of plant biotechnology built upon their indigenous capacity fortified by links with the CGIAR system and affiliated centres.

Progressively, the techniques of biotechnology are being applied to the major crops of the world, including wheat, maize, rice, potato, barley, cassava, soybean, sorghum, sugarcane, banana, tomato, cabbage, beans and mango. These techniques range from the relatively simple procedures associated with micropropagation to cell manipulations, coupled with plant regeneration from cultured plant cells and tissues. Cell manipulations centre upon the transfer of genes not only between sexually isolated plant species by protoplast fusion procedures, but also the transfer of genes directly into plant protoplasts, cells and tissues from other plant species, microorganisms or animal cells. The extent to which plant germplasm is being exploited and used in plant biotechnology is well illustrated with reference to the world's most important cereal, rice, and to tomato. Micropropagation is now being increasingly used to conserve wild rice species, and protoplast fusion procedures coupled with the regeneration of rice plants from protoplasts are being used to produce a novel range of improved cytoplasmic and nuclear combinations, including those for cytoplasmic male-sterility, and stress tolerances such as salinity tolerance. Direct gene transfer is being used to transfer genes for

enhanced insect and virus resistance. In tomato, plant regeneration from tissue cultures has been used to improve the sepctrum of germplasm, and protoplast fusion and plant regeneration from cultured cells used to facilitate gene flow from wild tomato species, with desirable features, into cultivated tomatoes. Overall biotechnology should not be viewed as a panacea for problems with existing germplasm but more as an oppotunity for the World to increase its biodiversity by accelerating and enabling novel germplasm production for the benefit of mankind.

INTRODUCTION

At this Symposium on Plant Germplasm Conservation-Perspectives for the 2000s, it is particularly timely, before discussing in detail the role of biotechnology in exploiting and using plant germplasm, to look back over the past decade as we attempt to look forward to the next. At the beginning of the 1980s, although it was clear that plant breeding using well established conventional methods would continue to make the key contribution to meet the demands of increased food production, it was forecast that multiple gene transfer mediated by somatic hybridization would, during the next few decades, assume increasing significance in the improvement of forage legumes, vegetable and fruit crops, It was also suggested that during the next decade it would be advantageous to attempt to incorporate into the recipient crop species some limited, perhaps single, genetic attribute of a donor species using a transformation procedure which would eliminate the need for backcrossing and recurrent selection. It was also forecast that in the more immediate short term practical gains would be made using tissue culture and protoplast cloning to enhance and release somatic variability in plants (Cocking et al., 1981).

Since 1981 most of these forecasts have been realised, but it has also been realised how vital plants and primitive crop varieties or 'land races' are to our ability to utilize these new methods of plant improvement for plant biotechnology. As long as we can protect these resources either by *in situ* conservation or *ex situ* conservation, there is hope that this partnership of modern science and the plant kingdom will flourish and continue to provide all we need for the crowded world of the next century (Huxley, 1984).

Plant cell, tissue and organ cultures

Plant biotechnology has greatly benefitted from the utilization of

methods which do not rely on sexual crossing or cross-hybridization, but which aim to propagate useful cultivars very rapidly and also create new ones with the help of plant cell, protoplast, tissue and organ culture, as well as genetic engineering techniques. Meristem culture and the regeneration of whole plants has been used for the micropropagation of cultivars and virus-free plants, and the culture of protoplasts and haploid tissues, somatic embryogenesis, selection of variants and mutants, protoplast fusion and regeneration of whole plants has been used for micropropagation and the improvement of cultivars. Genetic recombination, gene transfer and regeneration of whole plants have been applied to the improvement of cultivated plants and plant cell culture on a large scale for the production of useful substances (Sasson, 1989). The applications of these procedures to plant germplasm fall into three main categories: short-term applications which require about three years for their implementation, these include vegetative or clonal propagation; medium-term applications, such as the the development of somatic embryogenesis and embryo culture which require up to six or more years for the implementation; and long-term applications which cover gene transfer either through the production of somatic hybrid plants or transgenic plants.

The role of the CGIAR system and affiliated centres

The Consultative Group on International Agricultural Research (CGIAR) established in 1971 is an informal association of some 50 countries, international and regional organizations and private foundations working together to support agricultural research around the world. Today, the CGIAR is a global network of 16 international centres working to increase food production in less developed countries. Plant biotechnologies are increasingly being developed as effective procedures for the exploitation of plant germplasm within the limit of these various international agricultural research centres. At CIAT (the International Centre for Tropical Agriculture) plant cell, tissue and organ culture technology is being applied to improving beans, cassava and rice production for Latin America and the Caribbean, especially acid-tolerant upland rices. The International Maize and Wheat Improvement Centre (CIMMYT) conducts research and training related to the production of maize and wheat, two crops that together account for more than 40% of the cereal grains produced in the developing world. Because many of the procedures associated with the plant cell, tissue and organ culture of both wheat and maize have been more difficult than those for beans,

cassava and rice, progress in the application of biotechnology to the use of maize and wheat germplasm has been slow. At the International Rice Research Institute (IRRI) there is emphasis on the unique opportunities created by plant biotechnology to exploit the procedures of somatic hybridization and transgenic rice producation. The International Potato Centre (CIP) located in Lima, Peru, has taken the lead in collecting, developing and distributing potatoes adapted to the diverse growing conditions of the tropics, where the majority to the developing centres are located. The potato has been shown to be highly responsive to the various procedures for the application of plant cell, tissue and organ culture to potato improvement, utilizing the extensive germplasm of the World Potato Colection maintained at CIP. IRRI has similarly utilized its extensive germlasm collection of rice varieties, and in general, germplasm collections have greatly stimulated plant biotechnology research throughout the world.

The Syria-based International Centre for Agricultural Research in the Dry Areas (ICARDA) has world responsibility for the improvement of barley, lentil and faba bean and a regional mandate for the improvement of wheat, chickpea, pasture and forage crops. Largely because of technical difficulties associated with the development of plant biotechnological procedures for these crops progress has also been slow. A somewhat similar situation applies to the genetic improvement of sorghum, millets, pigeonpea, chickpea and groundnut (peanut) being undertaken at the Internaional Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and of cassava, maize, cowpeas, yams, soybean and plantain at the International Institute of Tropical Agriculture (IITA) outside of Ibadan, Nigeria. The International Network for the Improvement of Banana and Plantain (INIBAP) is particularly well poised to exploit the fact that procedures for the regeneration of banana plants via somatic embryogenesis have been established, (Teisson and De Langhe, 1989).

The exploitation and use of tomato germplasm

The tomato (*Lycopersicon esculentum*) owes its enormous commercial success to its wild relatives. The crop has gained resistance to the fungus *Fusarium oxysporum* from the wild relative *L. pimpinellifolium;* a change to the fruit stalk that adapts it for mechanical harvesting from another wild relative, *L. cheesmanii;* higher beta carotene from the wild relative *L. hirsutumand;* increased vitamin C content from another wild relative *L.*

peruvianum, (Huxley, 1984) The introgression of genes from these wild relatives has been achieved by sexual hybridization, sometimes, as for crosses involving *L. peruvianum*, using embryo rescue. The more extensive utilization of the germplasm of *L. pernvianum* has required the use of protoplast fusion technology.

Species of the genus *Lycopersicon* can be divided into two major groups on the basis of their sexual crossing relationships: a group of species that can be hybridised with the cultivated tomato with relative ease (the *'esculentum* complex'), and another group separated from the cultivated species by severe sexual barriers (the *'peruvianum* complex'). The species of the *'peruvianum* complex' represent a vast store of potentially useful genetic variation which tomato breeders are frequently deterred from utilizing because of the daunting sexual barriers which have to be overcome (Taylor and Al-Kumar, 1982). Gene transfer by protoplast fusion is an attractive additional procedure for transferring genes between members of the *'peruvianum* complex' and the cultivated tomato, and generally for the better exploitation of wild species of *Lycopersicon* because it overcomes the limitations of sexual isolation mechanisms. Protoplasts of *L. esculentum* can be readily isolated directly from tomato plants. The wild species *L. peruvianum* is readily capable of regeneration into plants from protoplast-derived callus (Zapata et al., 1977). Fertile somatic hybrid plants have been produced by the fusion of protoplasts of *L.esculentum* with protoplasts of *L. peruvianum* (Kinsara et al., 1986) and have led to an ability to readily introgress genes from the wild relative *L. peruvianum* into *L. esculentum*. Ongoing opportunity exists for the use of somatic hybridization to introgress other useful genes from other wild relatives of tomato from the extensive range of germplasm available.

Application of strict genetic engineering to a crop species such a tomato depends on the identification, isolation cloning and expression of genes of economic importance, whether these are genes for disease resistance, stress tolerance of improved yield potential. Cultivated tomato (*L. esculentum*) can be readily transformed using *Agrobacterium tumefaciens* as the vector and transgenic plants produced. Single genes can be readily transferred. As discussed by Sasson (1986), the transfer of genes coding for herbicide resistance has attracted financial support from several agrochemical companies, because of the commercial advantages resulting from the innovation. These include expansion of the market for wide spectrum and cheaper herbicides, to include crops previously susceptible, and extension of the period over which a company can continue to profit from the sale of its herbicides, even

after the expiry of patents, by selling them with the seeds of the resistant varieties. Resistance has been transferred to tomato by Holchst A. G.'s phosphinotricin and by Monsanto Co.'s glyphosate, the world's largest-selling herbicide. In addition to the transfer of genes coding for resistance to herbicides, the gene encoding *Bacillus thuringiensis* toxin has been transferred, and pest resistant tomato plants obtained.

In the future an evaluation will be required as to the relative merits of utilizing the natural biodiversity of germplasm available for particular gene transfers in tomato, as compared with the direct transfer of specific genes.

The exploitation and use of rice germplasm

Until recently the sexual input of genes from wild rice species into cultivated rice (*Oryza sativa*) was limited to one particular improvement, but, as recounted by Huxley (1984), with spectacular results. Following the Green Revolution with its associated large-scale paddies cropping twice a year, grassy stunt virus carried by the brown planthopper appeared almost from nowhere as an important disease. Screening the then 6,273 samples of rice kept in the collection of IRRI revealed only one, the feeble *Oryza nivara* that had the required resistance character; as a bonus, it was also resistant to blast, another major disease of rice. This sample of *Oryza nivara* had been collected from central India in 1966, at a time as highlighted by Huxley (1984) when noone would have guessed that it would ever be of value. Now thanks to *Oryza nivara,* IR36 the rice variety with improved virus resistance was once planted across more than 10 million hectares.*

Apart from *Oryza nivara,* wild species within the genus *Oryza* and other relevant Graminaceous species offer sources of important genes conferring traits such as salinity tolerance and resistance to pests and diseases (Chang and Vaughan, 1991). Since, as discussed by Finch et al. (1991), because many of the wild species in question are sexually isolated from cultivated rice, protoplast fusion offers a unique opportunity to develop previously unobtainable hybrids and cybrids, particularly in relation to novel cytoplasmic male-sterility. Furthermore, direct interaction of protoplasts with recombinant plasmids can lead to the incorporation and expression of specific -gene constructs. In this connection, to take advantage of the potential that

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* Note added by the editors: Since the early 1980s, the *Gsv* gene from *O. nivara* has been rendered ineffective by the appearance of biotype 2 of the grassy stunt virus.

array of characters that breeders are concerned about in population or variety improvement programmes must be mapped. To provide breeders with a useful repertoire of new tools, a saturated linkage map based on both molecular markers and phenotypic characters of agronomic importance must be available. As discuseed by McCouch and Tanksley (1991), as the current rice map evolves towards saturation, and new technologies give rise to new types of genetic markers and new possibilities for locating and locating genes of interest, the opportunities for effectively integrating molecular analysis of genetic variation into existing plant improvement programmes become increasingly apparent.

Protoplast fusion and transformation leading to the production of transgenic plants are techniques which have previously been limited mainly to the dicotyledons. However, recent successes in plant regeneration from protoplasts (Lynch et al., 1991) have enabled the coupling of protoplast to plant technology with recombinant DNA and fusion technologies, thus linking rice breeding and biotechnology. Finch et al. (1990) obtained dividing heterokaryons from fused protoplasts of the japonica rice variety Taipei 309 and mesophyll protoplasts of the important wild rice relative, *Porteresia coarctata*. The latter species is highly salt tolerant, but is sexually isolated from tribe Oryzeae; this work forms a basis for the somatic hybridization of these two important species providing the opportunity for gene flow for salt tolerance into cultivated rice. The first successes in the production of transgenic rice plants from protoplasts by direct interaction of plasmids with protoplasts were reported in 1988 for japonica rice varieties (Hodges et al., 1991). The transfer of a specific gene or group of genes into cultivated rice has a wide potential application in the genetic manipulation of rice. Potentially useful genes for rice genetic engineering include those for enhanced yield potential, pest resistance, abiotic stress tolerance, improved product quality, and lowered production costs (Toenniessen, 1991).

As for tomato and the other major crops of the world including wheat, maize, potato, barley, cassava, soybean, sorghum, sugarcane, banana, cabbage and beans, in the future an evaluation will be required for rice as to the relative merits of utilizing the natural biodiversity of germplasm available for particular gene transfers as compared to the direct transfer of specific genes obtained from other sources.

Cryopreservation and *in vitro* propagation

The seeds of most of the major crops are orthodox (nonrecalcitrant) and

can be refrigerated, and therefore in this regard cryopreservation in liquid nitrogen (-196°C) is of limited value. For some recalcitrant seeds, particularly those of semiarid lands, such cryopreservation is particularly useful. Cell cultures used in various plant genetic manipulations on repeated subculturing undergo genetic erosions, polyploidy, gene amplification and chromosome elimination changes; cryopreservation at -196 °C holds great promise to maintain their genetic stability. Such cryopreservation has, for instance, been very effectively used to maintain the plant regeneration capability of rice cell suspension cultures, and of rice protoplasts isolated from cell suspension cultures. This facility has helped greatly in enabing laboratories in developing countries to benefit from this technology transfer in the Rice Biotechnology Program of the Rockefeller Foundation (Lynch et al., 1991).

Clonal propagation of shoots *in vitro,* or micropropagation, allows the rapid multiplication of a particular plant species in a sterile, disease-free environment whilst minimising somaclonal variation, and is thus a particularly useful technique for the multipication or conservation of male-sterile or rare genotypes. An example is the recent development of a method for the *in vitro* clonal propagation of shoots from a range of wild rices, and other grass species, that have important genetic traits such as drought resistance and salinity tolerance (Finch et al., 1992). The axenic multiple shoot cultures could be maintained without subculture for several months, or rapidly multiplied, for the subsequent production of mature plants and seeds. It is envisaged that this clonal propagation technique could aid the genetic manipulation of cultivated rice by providing a means to vegetatively conserve valuable genetic resources, a technique to rapidly multiply novel hybrid material, and a source of embryogenic callus that will allow the application of biotechnological techniques, such as somatic hybridization and genetic transformation to perviously unexploited species.

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INTERNATIONAL COLLABORATION IN PLANT GENETIC RESOURCES: THE ROLE OF IBPGR

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ABSTRACT

Since its inception, almost 20 years ago, international collaboration has played a central role for IBPGR in carrying out its mission to strengthen the conservation and use of plant genetic resources. Such collaboration is necessary as IBPGR possesses neither genebanks nor research facilities.

An examination is made of the variety of forms and functions of present and future international collaboration, including: 1) a shift from IBPGR's direct involvement in collection and conservation in the early years towards collaboration with national programmes to assist them to conserve and use plant genetic resources (PGR) more effectively; 2) increased emphasis on a variety of network forms including regional networks and crop-based networks that encourage conservation and use of PGR among countries in a region; 3) focus on clarifying and advising on issues which may be constraints in collaboration including guidelines for the safe movement of germplasm, on policies regarding sovereignty of germplasm and intellectual property rights, formulating standards for more efficiently conserving germplasm in genebanks and developing standard systems for documenting germplasm accessions; 4) closer collaboration with other international agricultural research centres (IARCs) and other international organizations as centres for expertise and regional training on PGR; 5) strengthening the PGR component of existing networks such as the Bamboo and Rattan Network; 6) developing collaborative arrangements or contracting with universities in developed or developing countries, institutions to carry out research and development on topics such as genetic diversity study, management of landraces, community-based conservation, or recalcitrant seeds; 7) collaboration with donor organizations to secure funds for both IBPGR and national programme institutions.

The advantages of this collaborative mode of operation include

increased flexibility to respond to changing conservation needs and opportunities, and cost effectiveness in a world where PGR must be sustainably conserved in the face of limited funding.

Changes in IBPGR management and strategy, including increased decentralization to 5 regional offices will facilitate improved regional collaboration. The Asia, Pacific and Oceania region is particularly decentralized with a regional office based in Singapore and coordination offices in Beijing and New Delhi.

It is expected that IBPGR's emphasis on international collaboration should facilitate greater regional involvement and strengthening of such institutions as NPGRC.

An example is presented of the achievements from collaboration through the Regional Committee for South-East Asia (RECSEA).

EARLY ACHIEVEMENTS

The International Board for Plant Genetic Resources was founded almost 20 years ago, as a result of concern over the scale of genetic erosion and an increasing recognition of the potential value of the genetic resources that were being lost. The eloquence of advocates such as Frankel (1975) helped to establish how plant genetic resources could be conserved through the exploration, collection, conservation and documentation on a global basis. Thus, the broad mandate for IBPGR has always been clear to advance the conservation and use of plant genetic resources for the benefit of present and future generation (IPGRI, 1993a).

IBPGR was established as a small secretariat under FAO in Rome in 1974, but soon emerged as a sister centre in the Consultative Group on International Agricultural Research (CGIAR) system. From the beginning, IBPGR placed high priority to collect in areas and for species most threatened by genetic erosion, and to insure their conservation and documentation in a network of genebanks in international centres and national programmes around the world. Other early activities were focussed on promoting public awareness of plant genetic resources through meetings, publications and training. Support to research aimed at developing better techniques for conservation started during the first 5 years of IBPGR's existence.

The results of these activities in the recently published IPGRI Strategy (1993a), have been impressive.

- Almost 200,000 samples of germplasm have been collected on missions

sponsored by IBPGR.

IBPGR's scientific and technical support to national genetic resources programmes has contributed to the establishment in over 100 countries of *ex situ* conservation facilities comprising national and international storage facilities for germplasm of particular crops (Figure 1).

Standards for characterizing and documenting germplasm accessions were developed. Over 70 standardized descriptor lists were developed, which are widely used around the world. Seven volumes in a series of Directories of Germplasm Collections, listing the germplasm holdings of spectific crops held around the world, have been published.

Almost 1500 trainees on different aspects of plant genetic resources have been sponsored by IBPGR, 60% of whom continue to work on some aspects of plant genetic resources.

IBPGR has published over 200 publications including newsletters, research textbooks and conference proceedings.

Research supported by IBPGR resulted in seed storage methods and *ex situ* seed bank storage and management guidelines being developed. *In vitro* conservation methods for many vegetatively propagated species are also a practical reality. Collaboration between IBPGR and FAO on plant pathology and quarantine resulted in the publication of a rapidly growing list of widely used guidelines for safe movement of germplasm. Research on genetic diversity, conservation technology and regeneration, has resulted in significant advances in conservation strategies and techniques.

RESPONSE IN A CHANGING WORLD

Political, demographic, economic and environmental changes can affect biodiversity and plant genetic resources. The continued expansion in the world population, expected to reach 7 billion by the year 2010, with most increase expected in the Asian region, will require increase in food production never before achieved. Increased industrialization and climate change are likely to result in changes in agriculture. Plant genetic resources will play a key role in meeting the challenges posed by these changes.

At the same time, issues of accessibility and national sovereignty of plant genetic resources are topics of hot debate and concern, most recently as a result of the UNCED Convention on Bioldiversity signed by most countries of the world. Rapid advances in biotechnology can offer improved methods for collecting, conservation, measuring genetic diversity, disease indexing,

gene transfer and DNA storage. At the same time, there is an increasing trend in some parts of the world to apply intellectual property protection and other form of patents in order to restrict the availability of certain plants, genes or technologies in order to make their commercial exploitation more attractive.

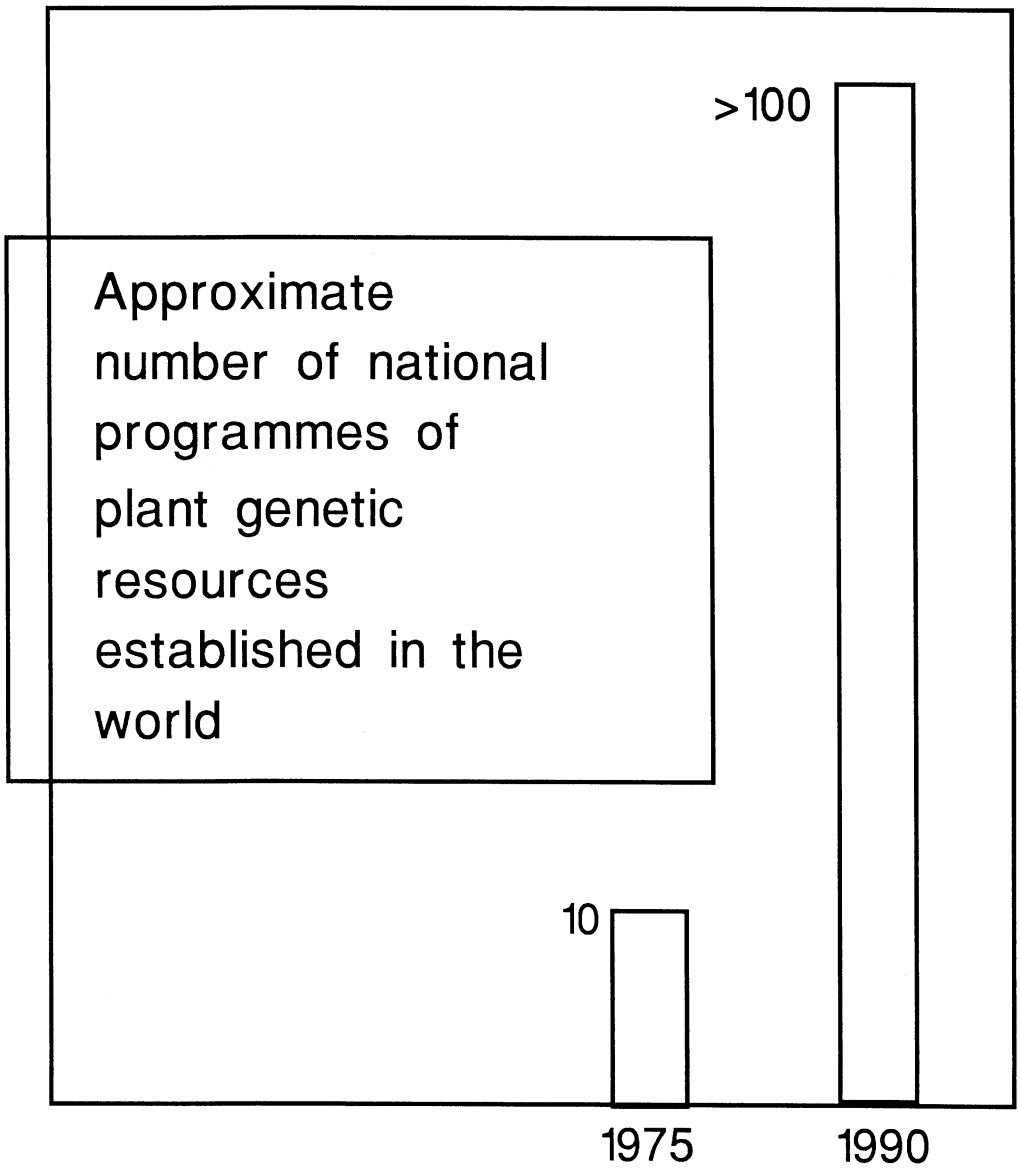


Figure 1. Increase in national plant genetic resources programmes.

These challenges are of critical importance both to IBPGR and to national programmes. Flexibility and responsiveness in addressing these issues are essential. IBPGR is now prepared to emerge as a fully independent International Plant Genetic Resources Institute (IPGRI) and has developed a strategy document (IPGRI, 1993a) and a medium-term plan up to 1998 (IPGRI, 1993b). These documents contain a framework for IPGRI's future programme and activities.

It is foreseen that IPGRI's core programme budget will remain very limited, but there are likely to be opportunities to identify sources of external funding and to develop collaborative activities with a number of partners.

The fact that IBPGR has moved away from directly controlling and organizing collecting, seed handling and gene bank storage puts the Institute in a better position to function as a true partner in collaborative activities.

PARTNERS IN COLLABORATION

The strategy document (IPGRI, 1993a) describes collaboration with a variety of partners including National Programmes, CGIAR centres and other international agricultural research institutes, United Nations agencies, regional organizations, non-governmental organizations, and the private sectors.

National Programmes

IPGRI's first and perhaps most important objective is "to assist countries particularly developing nations to assess and meet their needs for plant genetic resources conservation, and to strengthen links with users". Specific collaboration with national programmes can include: assisting in assessing the needs of a national programme regarding plant genetic resources conservation and use; advising on national priorities and plans, and national committees or coordinating systems; providing scientific, technical and policy advice; providing training opportunities; contracting of research projects to appropriate national institutes; assisting in rescue collecting; encouraging the adoption of standards in genebank management and quarantine; assisting in raising public awareness and in securing adequate funding for national PGR activities.

IPGRI accepts a global responsibility but recognizes that it will not be able to devote equal attention to all countries. Priority will be given to countries which: attach importance to the development of their forestry and

agricultural sectors; demonstrate a willingness to strengthen their own activities on the conservation and use of plant genetic resources; are willing to participate in regional or international efforts and, possess significant diversity of plant genetic resources.

The specific areas where collaboration between a national programme and IBPGR might be expected to develop is shown diagrammatically in Figure 2. This diagram emphasizes the importance of assessment of priorities and commitments of resources and capabilities by both partners. External funding may be required for some but, not necessarily all collaborative activities.

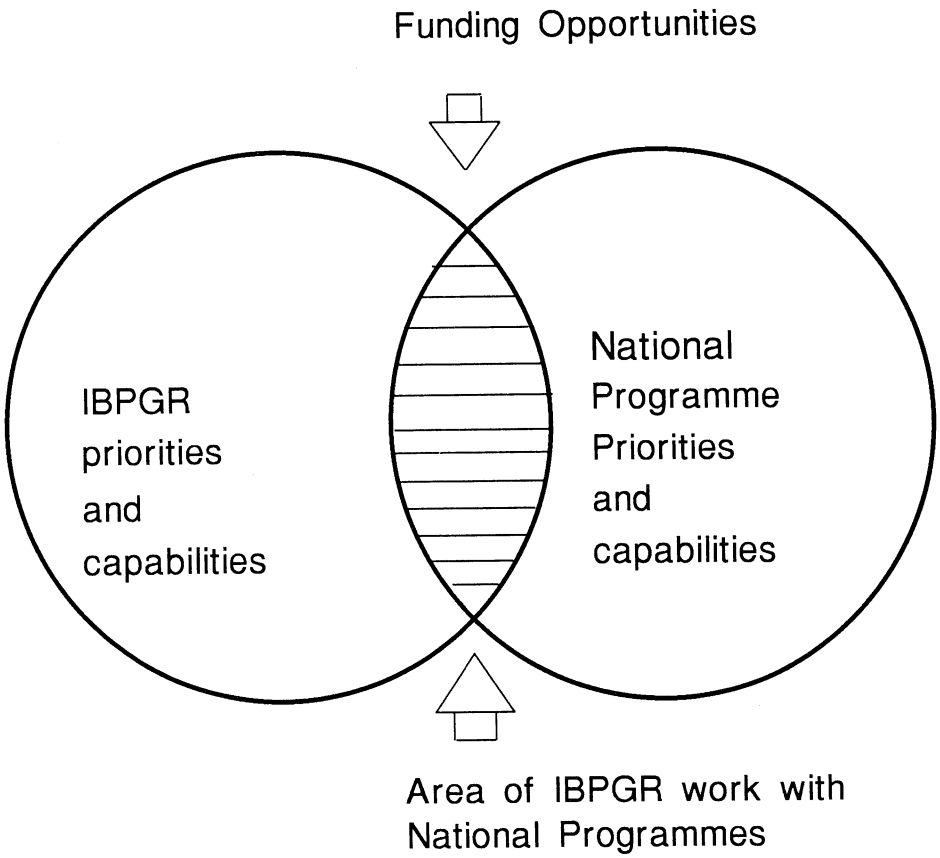


Figure 2. Identification of IBPGR work with National Programmes.

Consultative Group on International Agricultural Research (CGIAR)

The other International Agricultural Research Centres in the CGIAR

system such as IRRI, AVRDC, and ICRISAT collectively hold a large part of the world's *ex situ* food crop genetic resources. An Inter-Centre Working Group on Plant Genetic Resources provides a mechanism for collaboration among the centres. As these centres move toward partnerships with national programmes, opportunities for collaboration to involve IBPGR will increase. The newly created Centre for International Forestry Research (CIFOR) may develop linkages with IBPGR on a forestry genetic resources programme.

F. A. O. of the United Nations

FAO is a key participant in work on plant genetic resources in both crops and forest species. As IPGRI achieves independence as an institute, collaboration with FAO in implementing the International Undertaking on Plant Genetiç Resources will continue. A Memorandum of Understanding between FAO and IPGRI establishes clear roles for each organization for future collaboration. IBPGR will assist FAO to encourage countries to bring their collections into the International Network of Base *Ex Situ* Collections.

Regional Programmes

Regional programmes such as the Regional Committee (on plant genetic resources) for Southeast Asia (RECSEA), will continue to be important partners in collaborative activities. This is discussed below under the heading of Networks.

Non- Governmental Organizations (NGOs)

NGOs have made impressive progress in stimulating a critical examination of plant genetic resources conservation objectives and approaches by international and national organizations and by farmers themselves. IBPGR hopes to work much more closely in the future with such organizations. As an example, the regional NGO known as SEARICE and IBPGR plan to work together to better understand farmers and local users' strategies in conserving and using semi- and non-cultivated plant species in Laos.

Private Sector

Public and private seed companies, and commercial biotechnology companies may become partners with IBPGR in specific activities in the future to work on specific aspects of PGR conservation and use.

FORMS OF COLLABORATION

The second objective of the IPGRI strategy is "to strengthen and contribute to international collaboration in the conservation and use of plant genetic resources". Collaboration can take several forms.

Regional and Crop Networks are important mechanisms or tools for IBPGR that can bring together groups of people, organizations or countries to share information or other resources so that each derives greater benefit than had the resources been used in any other way.

Networks can vary greatly in complexity, but the identification of common priorities among members (Figure 3) can form the basis for effective and sustained collaboration. Generally a commitment for funding, and an agreement on one of the members, or a separate centre to function in a coordination role, or a "hub", is necessary.

Regional Genetic Resources Networks have been established in Europe, Latin America, Southern Africa, and West Asia and North Africa, and Southeast Asia. Regional networks allow countries within a region that share agro-ecological conditions to develop common activities on genetic resources. IBPGR staff can interact effectively as a member of these networks and often help play a coordination role. Work to conserve and use specific crops or commodity species can be carried out by working groups under these networks.

In this region, RECSEA can be used as an example of a regional genetic resources network. RECSEA first began functioning as a committee of IBPGR in 1975, bringing together country representatives from Thailand, Philippines, Indonesia, Malaysia and Papua New Guinea. With strong funding support, some impressive results were achieved. National Plant Genetic Resources Committees were formed in each participating country. Over 13,000 collections were made and shared among the 5 member countries. For example, a regional field genebank for bananas was estabished in the Philippines on behalf of all RECSEA members. A RECSEA newsletter (later to become the IBPGR regional newsletter) was established. Support channelled through RECSEA was used to establish or strengthen genebanks in each participating country. During 1984-1987, working groups were set up to address the conservation food legumes, tropical fruits, roots and tubers, maize, sugar cane, and coconut and oil palm. Posters were displayed and meetings were held to raise public awareness about plant genetic resources in these countries. As this committee makes the transition to a network,

IBPGR is working with RECSEA in assisting with communications, helping to seek

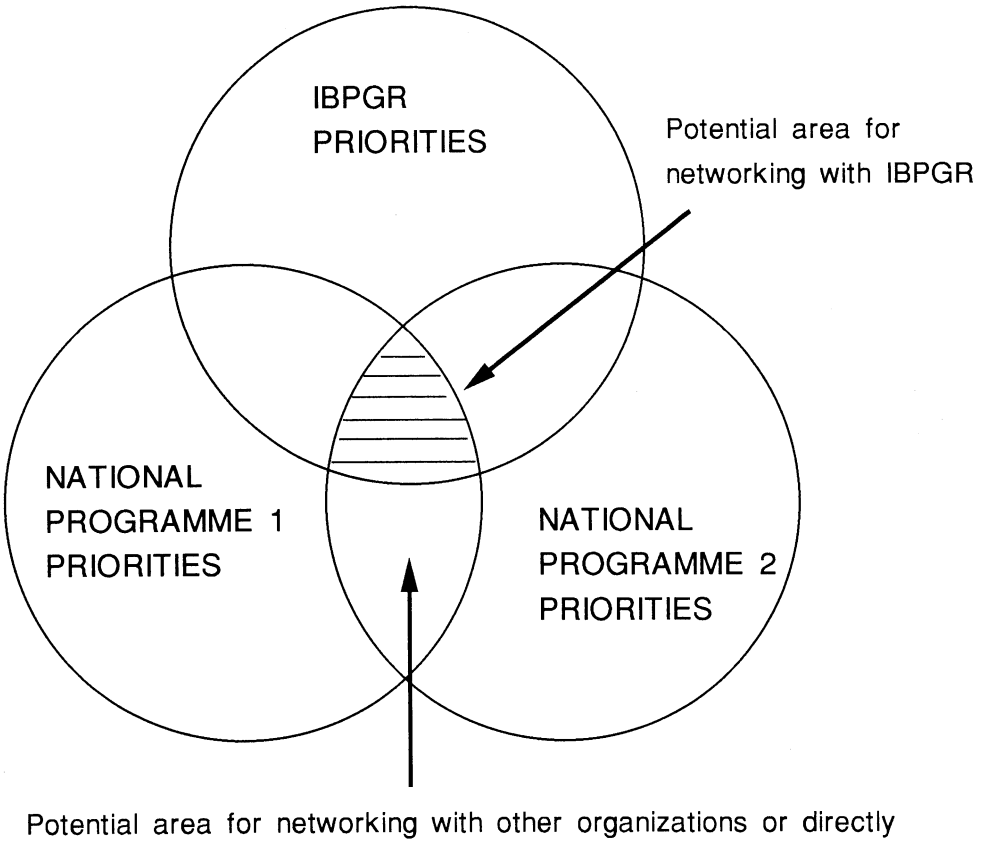


Figure 3. Identification of areas for networking between IBPGR and National Programmes.

funds and in making links with other IBPGR regional projects.

Crop Networks are concerned with the conservation, documentation and use of a particular crop, on a global or regional basis. A national programme. or in some cases an International Centre, acts as the coordinator. The development of a common database of the germplasm holdings of each participating country is seen as a necessary first step to enable further exchange and collaboration. Crop networks for beta, rice, okra, maize, barley, coconut, cassava, sweet potato, medics and groundnut have been initiated. IBPGR support was committed only for the initiation period for each of these networks. IBPGR is now considering how such networks might function more

sustainably.

IBPGR support may be given to help strengthen the genetic resources component of a network that is already functioning. For example the International Bamboo and Rattan Network (INBAR), now based in Delhi, is primarily concered with improving the utilization and production of these non-timber forest species. IBPGR is now set to provide complementary support for research in genetic diversity that can help in better planning of extractive reserves and *in situ* conservation.

Many of the research activities in which IBPGR is engaged can help both regional and crop networks function more effectively. Up-to-date information and advice can be provided on guidelines for safe movement of germplasm, the base collection network; standards for genebanks including "ultra dry seed technology" and regeneration; methods for conserving recalcitrant seeds or vegetatively propagated species; systems for documenting collections; methods for assessing genetic diversity and efficient collecting strategies and advise on policy and public awareness, including questions of sovereignty and intellectual property protection of germplasm or genes.

Research collaboration on new methods or techniques for the better conservation and use of plant genetic resources is an importatnt part of IBPGR's overall programme. On some topics, such as ultra dry seed, or methods to measure genetic diversity, many institutions around the world may be presently carrying out research, or have strong capability in these areas. In such cases, IBPGR can encourage information exchange and collaboration among these institutions and help guide the overall direction of the research to insure maximum effectiveness. Such collaboration can be termed " topic-based networking".

Funding is an important element to ensure continued progress in research and conservation activities. As mentioned earlier, IBPGR core funds are extremely limited, and national programmes and international Centres are expected to provide funding to conserve and use the germplasm that they hold. However, plant genetic resources is a topic of concern to many donor organizations. The increased awareness about the importance of conserving our global environment, particularly after the UNCED Conference and the Convention on Biodiversity, has resulted in greater potential for funding for national and international activities to conserve plant genetic resources. IBPGR is prepared to assist its partners in developing proposals to secure funds to carry out important collaborative activities.

ORGANIZATIONAL CHANGES IN IBPGR

As IBPGR prepares to become an independent institute, a number of organizational and management changes have been undertaken. The former Research and Field Programmes of IBPGR are now managed under 8 Groups. Three Groups focussing on thematic areas, namely Genetic Diversity; Germplasm, Maintenance and Use; and Documentation, Training and Information, are based in Rome, while 5 regional groups: Sub-Saharan Africa, West Asia and North Africa, the Americas, Europe and Asia, the Pacific and Oceania, have regional offices located within their regions, and are responsible for regionally-based activities.

Binding these Groups together are a set of projects in which all programme activities are funded and carried out. Each project involves at least 2 different groups and many involve the collaboration of 4 groups or more.

The Regional Office of the Asia, Pacific and Oceania Group is based in Singapore and now consists of 4 professional staff. Coordinating offices are located in Delhi and Beijing to look after activities in South and East Asia respectively. Regionally-based projects that have been developed now include national programme support, the coconut network, underutilized crops, tropical fruit trees, taro and yam, genebank regeneration studies, and support to strengthen other networks including bamboo and rattan, alliums, rice, groundnuts, okra and sweet potato.

Increased decentralization of the IBPGR programme, project set and activities is an improtant management principle. Increased responsibility to formulate and manage activities in the regional offices, is allowing IBPGR to move closer to its partners in developing truely collaborative activities with emphasis on responding to national programme needs.

With the strong emphasis on collaboration in the region from IBPGR and the exciting new developments in the strengthening of the plant genetic resources programmes in Taiwan, there are good prospects for closer collaboration among IBPGR, NPGRC and other organizations.

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THE HUMAN FACTOR IN PLANT GENETIC RESOURCES CONSERVATION

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ABSTRACT

Expansion of or establishment of plant genetic resources (PGR) programs by national and international agricultural research centers during the past two decades, coupled with vastly expanded field collection activities, has markedly increased the world's PGR collections. However, genetic erosion in farmer's fields, natural habitats and inside genebanks also continued at alarming rates.

A crucial component in conservation efficacy is the human factor: the quantity and quality of PGR workers. Many workers are inadequately trained or poorly prepared to accept the tedium, heavy load, multifaceted assignments, and low recognition related to their tasks. Their mission generally receives poor support from the administration.

As remedial measures, training programs of various formats need to be redevised and expanded to develop more competent workers for this young and growing technology. The morale of PGR workers needs to be boosted. The workers themselves also need to strive for greater interdisciplinary roles and improved service spirit so as to become more effective partners in the expanded crop improvement projects involving biotechnological approaches. Another crucial measure hinges on greater inter-institutional and international collaboration in fully using available resources. Schemes of collaboration are proposed.

Plant growers, whether farmers or home gardeners, should be enrolled to participate in conservation efforts. The public sector of human society should be better informed on PGR conservation so as to secure enhanced and sustained support for the necessary activities.

INTRODUCTION

During the past two decades the world has witnessed a keen awareness of the importance of plant genetic resources (PGR) and the need to conserve biological diversity. In the scientific community the recognition of dwindling biological resources in nature was intensified by the forays of scientific teams into little traveled areas where serious genetic erosion has occurred. Awareness of the general public was hightened by the growing concern for the quality of the human ecosystem. Reports of the mass media about the strife centering on germplasm ownership control and the use of this vanishing resources magnified the heated debates in the FAO sessions in the late 1980s. Greatly publicized controversies on the rights of use aroused by socio-political activists have thus helped to direct public attention to PGR which remained an adjunct to plant breeding until the late 1960s (Chang, 1985a: Frankel, 1985-87; National Research Council, 1993).

The merging forces led to greatly expanded and accelerated conservation activities in the 1970s and lasting through the 1980s, largely in field collection operations and the establishment of PGR centers (genebanks) by national and international institutions. Of these, the International Agricultural Research Centers (IARCs) under the Consultative Group on International Agricultural Research system, notably the International Board for Plant Genetic Resources (IBPGR) established in 1974, have played a leading role in collecting, preservation, dissemination and use of crop germplasm. Success of the IARCs has stimulated many national agricultural research systems to initiate or expand such activities. By the mid-1980s, the total holdings of the world's genebanks grew to 2.5 million accessions, of which a little over one million may be considered distinct or unduplicated in another genebank (see Chang, 1992). However, genetic erosion, both in the field and inside genebanks, also occurred at an alarming pace (see Chang et al, 1979; Goodman, 1984; Harlan, 1984; Fowler et al., 1988), though the exact statistics are not available. It is also open to question as to how many conserved accessions are viable and available for use.

Genetic conservation of plants is exclusively a human activity which dated back to the beginning of agriculture when humans extended help in plant propagation and perpetuation. Plants also need human inputs in the preservation of their genetic integrity and diversity. It was a mistaken notion of many people that plants can "take care" of themselves, while biodiversity has been on the decline since modern agriculture began. Nowadays even the

wild relatives of rice need human care (Vaughan and Chang, 1992). It has been pointed out that PGR conservation is a young technology and yet to be fully developed (Chang, 1985a). On the other hand, it is a labor intensive, complex, painstaking activity, requiring the inputs of diverse disciplines and professions. The lack of well trained workers was keenly felt when field collecting activities were greatly expanded in the late 1970s and much of the acquired germplasm materials awaited proper processing and preservation.

The paper aims to discuss the human factor in PGR conservation: its crucial role in the overall program, the constraints, the needs in human resource development, and future directions. These aspects are vital to enhancing the efficacy and sustainability of PGR conservation programs.

RECENT GROWTH IN PGR CONSERVATION PROGRAMS: PHYSICAL ASPECTS

In addition to the active field collecting activities of the past two decades, encouraging signs in the global expansion of PGR programs are indicated by two developments: establishment of national PGR centers and the construction of modern genebank facilities. The most significant developments came from two large and germplasm-rich countries: India and mainland China. In India, the former Division of Plant Introduction in the Indian Agricultural Research Institute was reorganized and expanded into the National Bureau of Plant Genetic Resources in 1976. A modern genebank is nearly completed with assistance from the USAID and several regional stations are being improved with aid from the UK. On the China mainland, a new Institute of Crop Germplasm Resources was established in 1978 and two large refrigerated seed storage facilities were built in the 1980s. The second one having long-term storage facility and laboratory complex was built with assistance from the Rockefeller Foundation.

During the same period, several IARCs built or upgraded their genebank facilities. In Southeast Asia, modern genebanks were added in Thailand, Philippines, Sri Lanka, Burma, Nepal and Kampuchea, often with funds and equipment from Japan. Japan and the USA added new structures to their national genebanks, while the R.O. Korea built a new facility. The National Plant Genetic Resources Center in R.O. China on Taiwan is one of the latest additions.

The marked growth in genebanks with medium-term and/or long-term storage facilities, esp. in the developing world, is shown below (Pluckneet et al., 1983; Chang, 1992).

Category	No. in mid 1970s	No. in 1991
Developed countries	16	25
Regional bank in a developed country	1	1
Developing countries	3	30
Regional genebanks in devloping countries	1	2
IARCs in developing countries	4	9

While the less fortunate PGR centers without the cold rooms in the developing world are faced with the most serious challenge in the need for frequent seed rejuvenation, many modern genebanks in the developing countries are also stressed by huge electricity bills, lack of spare parts and lack of local expertise in refrigeration technology/repairs.

CONSTRAINTS IN PGR CONSERVATION PROGRAMS

While every PGR center tends to boast on the recent growth in germplasm holdings, nearly all centers share the following constraints (see Goodman, 1984; Anon., 1987; Peeters and Williams, 1984; Chang, 1985b; Williams, 1989; Engle and Chang, 1991) :

(1) Insufficient technical staff and supporting service crew--the staff is often skeletal.

(2) Inadequate planting, processing and storage facilities and substandard practices. Field regeneration and seed processing following the acquisition of a modern storage facility presents a crushing work load.

(3) Under-funded and/or unstable support from the authorities above.

(4) Huge backlog of germplasm materials to be regenerated, processed and stored; equally bulky records to be entered in computerized data management systems.

(5) Heavy work load related to requests for seed/information, while the service role receives little credit in crop improvement.

(6) Poor communication/coordination with affiliates in crop improve-

ment, resulting in inadequate evaluation and under use of conserved germplasm.

(7)Inadequate *ex situ* preservation and viability monitoring methods for certain crops and little *in situ* conservation for many crops.

These constraints inevitably lead to continuing erosion within the plant collections and affect the long-term security of the conserved stocks. Restricted dissemination of germplasm materials and related information have impaired multidisciplinary evaluation and research, resulting in a low use of the vast resources for productive purposes (see Chang, 1985c, d, 1991a, 1992; Marshall, 1989).

DIMENSIONS OF THE HUMAN FACTOR

As stated earlier, PGR conservation is based on human inputs. In the long and myriad-faceted process of conservation, the PGR workers face a complicated series of operations: field collecting , multiplication or regeneration, characterization of preliminary evaluation, preservation, dissemination, data management, and, more recently, germplasm enhancement and informing the public. Much of the expertise in dealing with diverse plant species requires technical knowledge and experience that are species-, location and growth stage-specific, stemming from one's inherent interest and intuition--often missing in published work. It takes long years for a dedicated worker to develop into an effective PGR specialist. Continuity of personnel in key operations is also a critical factor in ensuring the effectiveness of genebank operations (see Chang et al., 1989). It is unfortunate that the crucial role of PGR workers in promoting the security and use of germplasm collections was often overlooked by the decision-makers. They can play a central role in overcoming the existing constraints in PGR programs (Chang, 1987; Chang et al., 1989).

While we recognize the crucial role of the PGR workers, what are the common constraints? As common to most new frontiers of research and technology, both quantity and quality are the principal problems. This matter in PGR conservation is compounded by the lack of institutions/programs that can provide the appropriate training to a large number of trainees/students over a diverse array of specialized subjects on a sustained basis. Agricultrual colleges, with the exception of the Applied Biology Department in University of Birmingham, offer no more than a single semester course each in PGR and seed physiology. Professor J. G. Hawkes and associates at Birmingham

initiated the MS and PhD courese in 1975 with substantial aid from the IBPGR, but the training site has no agricultural experiment station or a genebank. A number of short courses, largely supported by the IBPGR and devoted to field collection, have been helpful in developing field collectors, but they later confronted the equally challenging task of multiplication, characterization, storage and dissemination which they were poorly prepared. We at the International Rice Research Institute (IRRI) have held training courese (4-6 months at IRRT, plus collecting in home country) that combined the teaching of principles and hands-on practices, but such courses are costly and confined to the rice crop. Other IARCs have also held similar courses with shorter durations. Other international courses dealt with data management, *in vitro* techniques and clonal genebank management. The University of California has conducted two short courses (4-5 weeks) of international scope which provided an overview of PGR activities, but again the costs were high.

Over the years with IBPGR funding, more than 200 students have received graduate training and over 500 trainees attended short courses (Williams, 1984; Hawkes, 1985), but the numbers lagged behind the actual needs. It is also problematic to continue various training programs in the face of dwindling financial resources.

In my extensive visits to national PGR centers, I found out that a large number of the college graduates who became PGR officers took the positions not out of their own interest but because other preferred jobs (frequently plant breeding) were not available to them. A substantial number of the workers did not stay long on PGR work. Those who stayed on often complained of excessive work load without supporting labor force, meagre opportunity to conduct publishable research projects, and little help or encouragement from the officers above.

They felt isolated and left out of the main stream of research or breeding. As a service sector, their contributions to crop improvement were rarely recognized. As a result, the morale was generally lower than expected.

It is a more daunting challenge to serve as a genbank manager (curator) who has to cope with numerous problems, esp. the high power bills and uncooperative refrigeration technicians. In day-to-day work, he has to deal with the harried needs of an under-supported technical staff. Coordinating the time-restricted field work with lab. processing and storage is particularly challenging. Few managers stayed long on their posts in the developing world.

In my meetings with fellow workers and in my recent writings, I have urged the PGR workers to fully exert their expertise and service capability

in helping other crop scientists on various research fronts that involve novel germplasm. With good service comes the respect of the users. Meanwhile, I exhort them to be more active and aggressive in offering their materials and knowledge so as to become a full partner in interdisciplinary evaluation, research and germplasm enhancement. The more senior workers are urged to share their expertise with newcomers as a part of the on-the-job training. A PGR worker should also devote time to educate other sectors of society on the importance of germplasm. However, assistance and collaboration from colleagues in other disciplines and the plant growers in the field are also essential (Chang, 1985c, 1991a, 1992). Assistance and collaboration from the private seed industry toward a freer exchange of germplasm would also help the overall conservation efforts.

Outside the scientific community, the growers of plants, whether farmers or home gardeners, are often overlooked as powerful coworkers in field conservation. The farmers are crucial to the success of *in situ* conservation of primitive cultivars (land races) and traditional types of fruit tress, vegetables and floral species. Their collaboration should be sought by national PGR systems. Even the school teachers and children can play a part: in northern Thailand the schools make an annual planting of traditional upland (dryland) rice varieties as a part of the teching practice and help in perpetuating the colorful and diverse rices (Vaughan and Chang, 1992).

Seed Saver's Exchange and similar community groups in the US have served an effective role in conserving old vegetable and floral cultivars (see RAFI, 1986; Fowler and Mooney, 1990). Voluntary activities of the NGOs are taken on new significance as governmental actions cannot catch up with events outside experiment stations.

NEEDS IN HUMAN RESOURCE DEVELOPMENT

Since effective PGR workers are a rare breed, let us ask the question: what are the requisites? First, the person has to be a naturalist who cares about plants and other components in the ecosystem. The major subject in college education can be in any field of plant science: genetics, breeding, physiology, taxonomy, pathology, plant propagation. The new worker must have the inner drive to learn about and experiment with the plants under his /her charge in order to become an expert on the plant(s) and the adapted environments. Team work and the spirit to serve others are also essential. Above all, a dedication to conserve plants and to expand their usefulness is the mission of a promising newcomer.

A PGR worker must deal with large numbers, be it collected samples or field plots. Prior exposure to plant breeding practices will come in handy. Facility with computer equipment and software programs is indispensable. A strong background in classical botany will be equally helpful as familiarity with biochemical-microbial techniques.

During the two training courses on rice germplasm conservation held at IRRI, we noted that the workers from developing nations were so used to work in isolation that they were initially uneasy about our practice of placing them in pairs during field or lab exercises. While most of the trainees soon became used to this novel mode of operation, a few preferred to work alone. The trainees were impressed by the open door format of our Germplasm Center where any user can have access to the open databooks and ask for seed or information. They witnessed our "Service is our motto" in action. They were also exposed to our tradition in which a worker would voluntarily fill in for another when the worker in charge was absent. Perhaps such exposures will benefit the trainees more in their later career than the massive technical details that the mentors tried to force feed them in a short course. The contact with workers in other national program would undoubtedly enhance germplasm exchange and collaboration following their return to home countries.

On-the-job training and supplementary short courses should form a continuing component of staff upgrading. In my experience, the most capable workers came from student-assistants who began to work for the Center in their junior years. Early training and careful screening are imperative in developing effective PGR workers because the assignments require infinite patience, vigilance and accuracy.

To help the managers, I have prepared a chapter on genebank management that discussed current issues in management (Chang, 1991b, c) in relation to some of the problems encountered by national workers in rice genebanks (Engle and Chang, 1991). For many PGR centers, successors should be recruited in advance and nurtured in a staff development program (Chang et al., 1989).

FUTURE ENDEAVORS

In looking forward into the twenty-first century, the world will need more and better PGR workers who will care for the preciously fewer plants and to fully exploit their potentials. Their collaborators will extend beyond plant breeders and biotechnologists to include workers in animal science and

forestry. Their service spirit and cooperative attitude will count as much as scientific expertise. We must prepare for the attrition in number due to the tedious and unglamorous nature of the work.

In the face of dwindling resources on a world-wide scale nations, institutions, disciplines and various sectors of society must pool and share their available resources in implementing and ever-growing array of operations that are a part of the food chain in feeding the burgeoning populace. PGR will continue to serve as the hub of all plant research improvement activities. Full collaboration between highly industrialized, germplasm -poor nations and the less developed, germplasm-rich nations is particularly curcial.

I have expanded on a previous scheme to extend the collaboration on germplasm conservation, research, enhancement and use as follows.

Less developed countries serving as:	Developed countries serving as:
sources of genetic diversity and germplasm materials	sources of funding and sophisticated research
site for seed rejuvenation in home environment	*ex situ* preservation and distribution sites
site of *in situ* conservation field research sites	purification and disease elimination of stocks
	genebank training centers

Moreover, the IARCs should continue to serve as essential links in international activities. The private seed industry should also contribute funds to the conservation efforts from which it has been a beneficiary. Public support to conservation efforts and international germplasm exchange will be boosted by improved information and communication programs.

Within one country, different research and educational institutions should also pool resources and collaborate on PGR training. As a case example, the National Seed Storage Laboratory of the US was urged to serve as a training center for PGR workers in the National Plant Germplasm System and from abroad (National Research Council, 1988). The National Plant Genetic Resources Center of R.O. China is also expected to play such a role in the future .

It is my conviction that the future generations of mankind will continue

to benefit from the caring, pooling and sharing of plant genetic resources only when they collaborate and coordinate their inputs.

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GENERAL DISCUSSION

A panel was appointed by the Organizing Committee of the Symposium to moderate the open discussion. The panel members were: T. T. Chang (chair), W.J. Ho (of PDAF), Warren Kuo (of NTU), C.H. Sean (of TARI), C.T. Shii (of NTU), Samson Tsou (of AVRDC) and C.C. Tu (of TARI).

Dr. Chang opened the session and remarked that one of the major objectives in holding the Symposium as a part of the National Plant Genetic Resources Center Inauguration is to solicit and gather broad-based suggestions and comments which would improve the overall effectiveness of the national plant germplasm system in general and the Center in particular. The discussions would be one way for the participants to express their appreciation for the warm hospitality of the host, Taiwan Agricultural Research Institute. He urged the participants to freely speak on issues of interest to them.

The Chair first asked Dr. Ming-Jen Fan, head of the Plant Genetic Resources Laboratory in TARI, to provide the participants with an overall picture of the system's organization and operations and the operational roles of the Center within the system, so as to give focus to the open discussions that will follow. Dr. Fan reported as follows.

Report on the National Plant Genetic Resources System's Organization and Operations.....Ming-Jen Fan

The national plant germplasm system was conceived in 1986 at the 3rd National Science and Technology Conference, aiming to strengthening the acquisition, conservation and use of crop germplasm toward the development of technologies that would more effectively exploit the plant genetic resources in a systematic manner. The plan calls for an amalgamation of capabilities of all agricultural research institutions in the nation with the Taiwan Agricultural Research Institute (TARI) playing a nuclear role. The systems-approach would encompass introduction, exchange, preservation, multiplication or regeneration, evaluation, and use on a collaborative basis. A National Plant Genetic Resources Center would be established at TARI to integrate the various operations and to upgrade the conservation activities. In the system, the Council of Agriculture (COA) is the highest authority in setting guidelines through a Germplasm Advisory Council and

in providing funds for new operations and research projects. On the operational aspect, the Department of Agriculture and Forestry under the Taiwan Provincial Government, (PDAF) directs its subordinate research institutes and experiment stations in executing the collecting, multiplication preservation, evaluation and breeding activities. The system also involves other institutions engaged in plant germplasm activities outside the PDAF: Academia Sinica, universities and colleges, commodity-specific research institutes (sugar, tobacco, banana), and the Asian Vegetable Research & Development Center. About 20 agencies are involved, holding 67,000 accessions of various economic species. In addition, the Bureau of Commodity Inspection and Quarantine handles the plant quarantine measures of germplasm materials being imported or exported.

Within TARI, the Center is charged with the duties of:

(1) Planning and coordinating the collection, exchange, importation, storage, and data entry of plant germplasm in the country.
(2) Implementing data entry and information transfer of preserved germplasm materials.
(3) Carrying out activities related to germplasm material and information exchange between our country and other countries.
(4) Executing short-term, medium-term and long-term storage of seeds and conservation of clonal germplasm, serving as a site for duplicate storage.
(5) Coordinating and implementing multiplication or regeneration of germplasm.
(6) Coordinating characterization and evaluation of germplasm.

Research departments in the TARI such as Agronomy and Horticulture will cooperate and assist the Center in germplasm multiplication or regeneration, characterization, evaluation and in certain cases, field conservation. The Dept. of Horticulture will assist the management of clonally propagated crops at the three field repositories. The Dept. of Agricultural Chemistry will make quality and component analyses of certain crop species. The Departments of Plant Pathology and Applied Zoology will evaluate materials for resistance to diseases and insects (and other animals) respectively, and help on post-entry quarantine.

Other experiment stations of TARI, such as those at Chiayi, Fengshan and Kuanshi, will share their collections of fruit trees, root crops and other clonally propagated crops with the Center by using the latter as a duplicate preservation site.

Other agencies inside or outside the PDAF structure will deposit a set of their collections with the Center and assist on foreign acquisition, characterization, regeneration and field conservation. Crop advisory committees will be organized to plan and coordinate the various activities particular to a crop, while the Center will serve these committees.

The Center's physical facilities for preservation at the headquarters in Wufeng consist of (1) three seed storage rooms (short-, medium- and long-term), (2) tissue culture room, (3) cryopreservation area, (4) storage room for tubers, bulbs and rhizomes, (5) a field nursery for seed crops, and (6) screen-houses for clonal materials and for post-entry quarantine. The long-term storage area is composed of three vaults, maintained at -12 ± 2℃ and 30 ± 3% R.H., each holding 80,000 accessions packed in aluminum cans. The medium-term room (1 ± 2 ℃ , 40 ± 3% R.H.) can hold 250,000 accessions in aluminum foil envelopes. The short-term room (84 sq.m. floor space) kept at 10 ± 2℃ and 40 ± 3% R.H. will hold working collections of various crops. The Center also has a state-of-the-art mini-computer system. It is capable of international link-ups.

At three outlying sites and at different elevations (300-500m, 900m. and 2350m.) clonally propagated crops will be maintained. The materials include temperate-zone fruit trees, forage grasses, medicinal plants, and woody flowering species.

With the above conservation facilities and excellent research labs at Wufeng, staffed by six scientific officers (incl. the head of Lab) and technicians, the Center will carry out the following scientific functions:

1. Serving as the national preservation center for plant germplasm.
2. Serving as an exchange center for plant germplasm.
3. Serving as a plant germplasm information service center.
4. Developing new preservation and identification techniques.

The Center's short-term goals (1993-95) are envisaged as:

1. To establish operational and management procedures of the germplasm bank, including standard operating procedures in monitoring seed viability.
2. To establish screenhouse facilities for quarantine and clonal propagated plants, to complete the field repositories for clonal germplasm.
3. To collect existing germplasm in the country.
4. To complete the germplasm information management system, and to establish access to information centers in other countries.

5. To store 30,000 accessions in the long-term vault.

The medium-term goals (1996-2001) are set as follows:

1. To complete the collection and preservation of native plant species and to proceed with field collection or acquisition of foreign germplasm.
2. To complete the consolidations of characterized data of various crops in the country.
3. To complete the base-line data files of major crops.
4. To complete the computer linkup and data inquiry system among various agricultural research institutions in the country.
5. To store 80,000 accessions in long-term vault.

In time, and with added staff capability and increased collaboration from domestic and foreign institutions, the Center will expand the international activities, aspiring to become a regional germplasm center for subtropical and tropical crops. Further details about the national system and the NPGRC may be found in a bulletin by Tu and Fan (1992).

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Tu, C. C. and M. J. Fan. 1992. The organization and operational goals of the National Plant Genetic Resources System of R.O.C.(in Chinese). 29 pp. Special Bull. 39. Taiwan Agricultural Research Institute, Wufeng, Taichung Hsien, Taiwan, R.O.C.

Open Discussion

Dr. C.O. Qualset urged the NPGRC to fully collaborate with the Taiwan Forestry Research Institute and other government agencies in biodiversity conservation. For example, the Center could help preserve forest tree seeds by providing duplicate storage. A similar arrangement

with AVRDC should be formalized. The Center could also join national parks in the seed conservation of endangered species and in monitoring populations in *in situ* conservation sites. It is important for an agricultural center to show interest in the broader environmental issues facing Taiwan. Moreover, Taiwan, and the Center in particular, has ample opportunities to partake international leadership in PGR activities, esp. for on-site training. With the fine training facilities and a wide array of scientific disciplines, TARI should actively participate in PGR training programs and recruit visiting scientists to supplement the present staff. The PGR training activities at U.C. Davis could cooperate with TARI by offering courses at the NPGRC and jointly seek funds. In both research and training, TARI could invite scientists from universities, affiliated research institutions and other departments of TARI to provide technical advice on plant-specific subjects or to conduct joint research on crop germplasm. Such complementary arrangements can enhance the scope and diversity of scientific expertise in the national system at relatively low cost.

Dr. L.M. Engle suggested that all those institutions holding crop germplasm should make use of the excellent facilities of the NPGRC, esp. the long-term storage space. Dr. Chang added that the collaborative ventures mentioned above would also develop comradeship and mutual trust among PGR workers of over 20 institutions in Taiwan that are affiliated with the national PGR system. Dr. Fan responded that the Center is taking in seeds conserved by the AVRDC and soon of the Forestry Research Institute.

Dr. M. Nakagahra asked in what manner does the TARI plan the operating budget, personnel and research projects of the Center. Director Tu answered that the operating needs and maintenance costs will be funded by annual allocations from the PDAF while research projects will be funded by the COA, PDAF or the National Science Council, of which the COA provided over 80 percent of the research needs in past years. Dr. Nakagahra stressed the need to plan from the level of the curators, both genebank and crop-wise. Dr. Tu concurred that much needs to be strived for in mobilizing scientific manpower from various agencies in Taiwan.

Dr. H.L. Shands stressed the need for close cooperation and adequate funding in the areas of regeneration and evaluation. He inquired about the plan to obtain such cooperation. Dr. Fan answered that various plant breeders in affiliated institutions and the AVRDC will assist the Center on regeneration, and assistance on evaluation will come from a broader array of scientists. Funds for joint activities will be sought from the COA as

needed.

Dr. E.E. Roos suggested that with the Center's excellent facilities in cryopreservation, though not fully developed, research on cryogenic storage should be a primary focus on research since emphasis has been placed on tropical and subtropical crops, many of which have recalcitrant seeds. Such research can also be implemented by another agency with support from the Center. However, he has concern about the availability of funds nowadays. Dr. Chang suggested a multi-agency approach to seek the necessary funds.

Dr. Warren Kuo proposed that the Center should collaborate with the University of Reading in the U.K. and the National Seed Storage Laboratory of the U.S.D.A. on seed preservation research. He also urged the Center to join IBPGR'S networks.

Dr. Ken Riley responded on the question of IBPGR funding of PGR research as follows. "The IBPGR is interested in getting involved by helping national programs to seek funds for well formulated research purposes. We are optimistic about the possibility of finding donors and providing those collaboration partners having clearly defined goals and the capability to attain the desired outputs. If such outputs can clearly improve PGR conservation, finding donors is likely to be easier".

Dr. Shands inquired about the discussions to-date that will bring together, either regionally or globally, genebank managers and program leaders to cooperative activities or to resolve areas of contention. Dr. Riley answered that this matter has been discussed within the IBPGR and at the South Asian regional level. The Southeast Asian group, known as RECSEA, has also met to discuss national PGR strategies including genebank management issues.

Dr. C.T. Shii inquired about the procedure of choosing priorities for preserving the germplasm of different crops. Drs. Tu and Fan answered that the guidelines will come from the National Advisory Council on Germplasm and the respective crop advisory committees, which are yet to be organized. Workshops will be held to bring together the workers of different agencies to discuss common issues. On a related question about the annual evaluation of PGR projects, Dr. Chang pointed out that for those projects carried out by agencies under the PDAF, the PDAF has a Research Review & Advisory Council to make annual approval and review of individual projects. It is a regular process of long standing. On the subject of research directions, Dr. C.T. Shii urged the NPGRC to emphasize the flora native to Taiwan. Dr. C.H. Sean suggested the medicinal plants be given attention. Dr. C.H. Hu (of Taichung DAIS) commented that

unless active research is a part of the Center's operations, it would be difficult to attract promising young scientists to join the Center or its affiliated projects. Several participants again emphasized the need to involve visiting scientists and collaborative research projects. Dr. Tu remarked that the first duty of the Center is to process conserved seedstocks into the long-term storage vault, though he endorses the need for research.

Dr. Samson Tsou urged the Center to enter into collaborative ventures with the International Agricultural Research Centers in the CGIAR system to strive beyond the roles of a national center and to engage in activities that will make the Center an active member of the international PGR community. Dr. Riley commented that active on-going research at the NPGRC will help it to become a regional training center. The IBPGR welcomes the Center's staff to participate in IBPGR-organized training courses on genebank management. IBPGR also plans to hold courses on *in vitro* conservation and on documentation.

During the course of questions and answers on the Center's operations, several participants pointed out that the present staff could barely manage the routine operations of conservation. Comments from the concerned participants pointed to the Center's need to develop well-defined annual work plans, a staffing pattern and staff development plan, and plans for expanded collaborative ventures in research and training.

Deputy Commissioner W.J. Ho of the PDAF thanked the invited speakers for their interesting and helpful presentations on various scientific aspects of PGR that will be useful to the national system. Thanks were also extended to the participants present for their valuable suggestions and comments. She assured that the PDAF will study and favorably consider the various proposals.

Recommendations and Resolutions Offered by the Participants of This Symposium:

Dr. Chang presented to the participants present a set of proposed recommendations and resolutions that were prepared by a 12-member drafting committee during lunch time. He pointed out that though the statements were brief, they have embodied the spirit and good intentions of the open discussion. It was also recognized that the TARI has already taken action on several issues.

The proposed points were read aloud and discussed. After a number

of participants gave specific comments or points of re-emphasis, the recommendations and resolutions were edited, approved and adopted by those present. The set was presented to TARI Director C.C. Tu as an outcome of this Session.

Final Recommendations and Resolutions:

1. The participants congratulate and commend the Council of Agriculture, the Taiwan Provincial Department of Agriculture & Forestry and other institutions involved in establishing the National Plant Genetic Resources Center at Taiwan Agricultural Research Institute (TARI). The new Center will go a long way in ensuring the supply of rich crop germplasm, both domestically and internationally, to the users.

2. In view of the immense operational scope and long-range goals of the Center, we, the participants, urge the appropriate government authorities to fully sustain the activities of the magnificent Center on both financial and administrative grounds. It becomes obvious that the Center will need increased staff and well trained scientists to augment the present staff. A staffing pattern and schedule in relation to the program of work should be developed and approved by the Department of Agriculture & Forestry. Similar budgetary planning should be made.

3. We urge the Center to seek assistance and cooperation from all agencies concerned in fulfilling its mission. Frequent consultations, periodic visits and meetings, and participation in events of an international nature will be helpful in securing the necessary collaboration. On the other hand, the Center should make germplasm materials and information freely available to all users.

4. Collaboration and coordination on both the domestic and international level is imperative to upgrade germplasm acquisition and exchange, duplicate storage of germplasm, staff development, data base development and link-ups, research on germplasm, and evaluation and use of the conserved germplasm. Research should receive high priority, esp. for subtropical and tropical crops, using cryopreservation. Research and technology development should be integrated with educational programs. Electronic database link-ups should be both national and international. In time, TARI should expand its training capability, even of an

international scope.

5. In order to guide and help the national PGR system, a national advisory council needs to be appointed to set guidelines on policy matters. Crop-specific advisory committees need to be organized as soon as possible, to provide interinstitutional link-ups and collaborative field collection. Standard operational procedures and sets of crop descriptors should be developed and adopted through the crop committees.

6. We urge all agencies concerned to strengthen their efforts on informing the public about the importance of PGR conservation through multi-channel media and educational programs.

7. We appreciate having the opportunity of taking part in this Symposium and we pledge our willingness to contribute to the operations of the Center and indirectly to the world community of plant conservationists and their clients.

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M. Nakagahra	K.W. Riley
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MESSAGE OF THANKS

Mr. Chairperson, distinguished guests, invited speakers, participants, ladies and gentlemen:

It is my great pleasure to represent the Organizing Committee to congratulate the success of inaugurating the National Plant Genetic Resources Center and the International Symposium on Plant Germplasm Conservation -- Perspectives for the 2000s. Foreseeing the importance of germplasm conservation, a national project "The Establishment of Plant Germplasm Bank and Enhancing the Use of Plant Germplasm" came into existence with the full support of the Council of Agriculture. TARI had been chosen to implement this project. On this special occasion, the Symposium not only celebrates the inauguration of NPGRC, it also aims to promote future international collaboration.

It is our honor to have 7 distinguished guests from the USA, United Kingdom, Japan and the IBPGR with us here. They are joined by three eminent speakers from institutions in Taiwan. They have discussed policy highlights and recent research activities on plant germplasm worldwide. During the past two days, excellent presentations and active discussions had made the Symposium a memorable event in the history of NPGRC of the ROC.

I would like to express my sincere gratitude to all invited speakers, especially those coming from abroad: Drs. Shands, Qualset, Nakaguhra, Bird, Roos, Cocking and Riley, who had taken long trips to join this Symposium and the inauguration of NPGRC. Special thanks are due to Dr. T.T. Chang, the President of SABRAO, who also is the most eminent leader among gene bank managers in the world. He had given us so many useful advices in constructing and operating the NPGRC. Thanks are also due to all members of the Organizing Committee who came from National Taiwan University, National Chung-Hsing University and AVRDC as well as many other institutions for their support of this Symposium.

Finally, with your full support, the NPGRC of ROC can play an important role internationally regarding plant genetic resources.

Once again, I hope all participants are satisfied with our arrangements and all our foreign guests have a pleasant stay in Taiwan. Thank you all.

C.C. Tu
Director, TARI

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