Chapter 7

Maintaining Plant Diversity Offsite

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HIGHLIGHTS

. Seed storage techniques are being used to conserve the genetic diversity of cereals, legumes, and many other important crop species. Some plants, however, do not produce seeds that can be stored. Eventually, this problem may be resolved with techniques for in ***vitro*** storage of plant tissue, from which whole plants can be regenerated. At present, the main alternative to seed storage is to grow entire specimens in the field.

- New technologies, including cryogenic storage of seeds and clones, use of biochemical methods to characterize accessions, and improved methods to detect pathogens in plant materials transferred internationally, have the potential to increase the cost-effectiveness of maintaining plant diversity offsite. Progress, however, is constrained by a lack of fundamental research on plant physiology, reproductive processes, and the mechanisms of genetic and cellular change.
- Priorities and protocols for collecting and maintaining germplasm of major crop plants are internationaIly coordinated. However, they are not well-organized for minor crops or for wild plants that are endangered or have economic potential.
- Long-term public and private support for germplasm storage facilities depends on whether the stored materials prove to be valuable. The use of offsite collections can be improved by characterizing the genetic diversity contained in the collections and evaluating collections for important traits.

Major breakthroughs in biotechnologies might eventually lead to fundamental changes in how biological diversity is maintained. Even so, a large portion of public resources for technology development should be used in improving the application of existing technologies, such as cryogenic storage of germplasm, that are important to society but are not attractive to the private sector to support.

OVERVIEW

One approach to maintaining plant diversity involves collecting samples of agricultural and wild species and storing them in offsite collections, Such collections can assemble agriculturally, geographically, and ecologically diverse plants for use in crop improvement, genetic research, or plant conservation. This chapter assesses technologies of collecting, storing, and using plants offsite,

Objectives Offsite Collections

Offsite collections of agricultural crops bring together varieties and related species from widely dispersed areas (box 7-A). These collections conserve plant genetic resources threatened with loss or extinction. They also serve as a convenient source of new genes for public and private plant improvement. The highly suc-

Box 7-A.—Definitions Relevant to Offsite Plant Collections

Most offsite plant collections are devoted to assembling a diversity of agricultural species, although collections exist for wild species (69,112). Offsite collections of agricultural crops can be classified according to their principal goals.

- **Base collections** focus on long-term preservation of genetic diversity to ensure against loss of valuable plant germplasm. Base collections often include crops and their wild relatives, typically maintained as seeds in long-term, subfreezing storage. The base collections important to agriculture in the United States are held at the National Seed Storage Laboratory in Fort Collins, Colorado, a part of the National Plant Germplasm System (NPGS) (see ch. 9).
- **Active collections** provide public and private plant breeders and researchers access for use in crop improvement, pharmaceutical studies, taxonomic investigations, or genetic research. Long-term security for plants in active collections is provided by duplicating their holdings in base collections. Within the NPGS, the Regional Plant Introduction Stations maintain active collections for numerous crops.
- **Genetic stock collections** constitute an array of plants with one or more unique genetic characteristics. These collections provide references for gene nomenclature and genetic mapping studies. Leading research institutions maintain many of these collections.
- **Working collections** are held by public and private plant breeders and contain many interrelated lines that serve crop improvement activities. Working collections are commonly obtained from active collections when new characteristics (e.g., resistance to a new disease) are needed for crop improvement. The genetic diversity in working collections is frequently limited to the specific goals of an individual breeding or research program.

Many of these collections, regardless of their specific goals, include a wide variety of accessions. Accessions in offsite plant collections can be classed as wild species, landraces, breeding lines, agricultural varieties, and engineered lines (18,46,52,103,115,116):

- **Wild species** related to agricultural crops are of particular interest because they may provide new and valuable gene complexes. Others may prove to be valuable sources for industrial, forest, and pharmaceutical products. But the bulk of wild species have no immediately obvious use. However, offsite maintenance becomes important when such species are endangered in their natural environment.
- **Landraces** have developed through generations by farmers who selected plants with the characteristics they desired. They represent populations that can be genetically diverse and are often specifically adapted to local environments and are considered to be valuable sources for the genetic traits that have enabled them to survive. However, a landrace may be productive in one region, but because it is so highly adapted, it may not grow well in another.
- **Breeding lines** are intermediate forms produced in the process of developing new agricultural varieties. Although saving all such lines would be impractical, some have permanent value to breeders (46). Probably most valuable of the breeding lines are those from which modern agricultural hybrids are produced.
- **Agricultural varieties** (cultivated varieties or cultivars) can include those under cultivation as well as those no longer in popular use. These generally are the products of breeding and development programs. If still widely being cultivated, agricultural varieties are often not collected or stored, because they are considered easily available.
- **Engineered lines** are the products of modern biotechnology. These are plants produced by methods of cell or tissue selection in culture, culture-induced mutation, or genetic engineering. Such materials are generally of more concern to scientists than breeders. The promise of transferring traits, such as nitrogen fixation or improved protein quality, may make such varieties important. At present, though, engineered lines are of minor concern to offsite collections.

cessful rice variety 1R36 developed by the International Rice Research Institute resulted from the crossbreeding of 13 accessions in their collections of rice from the United States and several Asian countries (Pg). wild plant collections help to preserve endangered species, supply materials to restore degraded lands, and provide material for genetic improvement of crops.

Botanic gardens and arboretums aretheprimary repositories for wild plant species *(69,* 112). Arboretums have been particularly important for maintaining individual trees and shrubs that may have little commercial significance *(69).* These facilities may also have commitments to public education and display that can result in selecting plants with special or unusual characteristics rather than those representing the genetic diversity within the species. However, many such institutions are now giving greater attention to the potential contributions they can make to maintaining plant diversity (12,34),

Considerations in Selecting Technologies

No single technology is appropriate for all the plants stored in offsite collections. Several considerations affect the selection of appropriate technologies such as biological limitations of the species, reliability of the technology, and cost.

Biological Limitations

Seeds are the most commonly and easily stored propagules of plants. When placed in conditions that reduce their moisture content to approximately 5 to *6* percent, seeds of many species will remain viable for years. Lowered temperatures can further extend storage life. Seeds able to withstand reduction in moisture and temperature are called ***orthodox*** seeds. Most of the major food crops (e.g., cereals and legumes) have orthodox seeds, and many, when properly dried, withstand cooling to – 196° C, the temperature used in cryopreservation (58, 85,89,100,108).

Seeds that cannot survive a reduction in moisture content are called recalcitrant seeds. Recalcitrant seeds are found in many important tropical species, a few temperate tree species, and some aquatic plants (8,42,83,100,108,118).

Reducing the water content of recalcitrant seeds severely shortens their life span. Thus, they cannot be stored like orthodox seeds: cooling to subfreezing temperatures would lead to

Photo credit International Board for *Plant* Genetic Resources

Many temperate and tropical species such as coffee and oil palm have seeds that cannot be stored for long periods; for this reason, collection of woody cuttings is preferred.

formation of ice, resulting in damaged cells and death of seeds. Instead, plants with recalcitrant seeds are commonly stored as field collections. Research on the physiology of recalcitrant seeds may lead to methods for long-term seed maintenance of these species. In vitro plantlet or embryo culture, coupled with cryogenic storage, may also eventually become useful for these species.

Two other biological limitations may restrict the maintenance of some plants. First, the qualities that distinguish a particular variety (e.g., flower color and shape in roses; or the color, flavor, and texture of a peach) may not be preserved in plants grown from seeds, For many fruit and nut varieties, retention of these specific qualities is only possible through clonal propagation, which entails producing plants from cuttings or by grafting. Second, some plants do not produce seeds readily because of inappropriate environmental conditions, physiological barriers, or genetic inabilities, In some cases, such as many varieties of banana or the tropical yams (Dioscorea), plants are sterile and seeds cannot be obtained. Basic studies of physiology are needed to improve understanding of the processes controlling flowering and seed production for both cultivated and wild plant species.

Reliability

The reliability of technology refers to both the potential for loss (by natural causes, accident, or equipment failure) and the likelihood of genetic alteration during storage.

The potential for loss by natural causes is higher in field collections than in seed collections. Pests, diseases, and environmental conditions can decimate field collections. Therefore, collections should be duplicated in different locations to ensure against loss. Greenhouses or other controlled environments may reduce the potential for loss by reducing environmental exposure. Research of in vitro culture may lead to alternatives to field collections that are free from disease and environmental uncertainties.

Collections are also subject to equipment failure. As a backup measure, mechanical refrigeration compressors should have alternative power systems to prevent warming, which may adversely affect the viability of stored seeds *(100).* Cryogenic techniques do not rely on external power sources or mechanical cooling systems. And though containers may develop leaks, the risks to security are considered much less than for mechanical refrigeration *(100,101).*

Some novel approaches to reducing dependence on mechanical cooling systems are being tested. The Nordic Gene Bank in Sweden recently established a long-term storage facility in old mines dug into the permafrost *(125).* The Polish Government proposed establishing a world seed collection in Antarctic ice caves, but this approach raises questions about storage temperatures and about ease of access and political control (55). An approach being developed in Argentina is to use the cold nights of mountain environments to cool a specially constructed storage vault (55). These options, while interesting, are suitable for only certain countries and are still experimental.

Genetic stability of plants can be affected in several ways. Mutations in orthodox seeds may increase as samples lose their ability to germinate *(84,108).* Growing out samples subjects them to conditions that will select against certain individuals and thus reduce genetic diversity in the sample (88). Cryogenic storage can improve genetic stability by slowing viability loss and lengthening the time needed between regenerations. For in vitro cultures, genetic mutation becomes a concern when the tissues are growing as unorganized calli. Cryogenic storage of such cultures could suppress mutation by arresting growth, but more research on plant development and cryopreservation of in vitro cultures is still needed *(90,108).*

Costs

A final consideration in the selection of technologies is cost. In seed storage facilities, expenses are incurred with monitoring viability and regenerating samples. Mechanical refrigeration systems can be expensive because of continuous energy costs. Cryogenic storage can lower long-term costs by reducing the fre-

quency of viability monitoring and regeneration. However, cryogenic storage is economical for only certain plant species; larger seeds, such as beans, may be stored more economically under mechanical refrigeration (102).

In vitro cultures may be more economical than extensive field collections, particularly if the cultures are stored cryogenically. However, further research and development is needed on both in vitro culture and cryogenic storage,

COLLECTING SAMPLES

Collecting samples involves the development of strategies as well as the actual collection of plants. Developing strategies can be facilitated by analyzing plants already in storage, Ideally, a strategy would provide for the collection of all genetic variants of a species without redundancy (37) (box 7-B).

Strategies

Considerations of economic or esthetic importance, rarity, degree of endangerment, access, genetic diversity, and similarity to plants already stored offsite can all influence the setting of strategies.

For the major agricultural species, collection strategies have been established by considering the data on plants already collected, geographic distribution of crop species, particular needs of breeders, and the economic or social importance of the crop *(117).* The International Board for Plant Genetic Resources has established a system of priority ratings to guide collectors: priority 1 crops are those with most urgent global collection needs; Priorities 2, 3, and 4 indicate descending orders of urgency *(51,73),*

Strategies are less clearly established for the collection of most wild species *(66),* In general, those threatened in their natural environment or of display value have received greater attention (69,1 12). A formal system for coordinating conservation activities has only recently been established (34,112). Botanic gardens, commercial institutions, and private collections have been growing and propagating rare plants for years but without concern for obtaining a range of genetic diversity (69). organizations such as the Botanic Gardens Conservation Coordinating Body of the International Union for the Conservation of Nature and Natural Resources (IUCN) and the Center for Plant Conservation at the Arnold Arboretum of Harvard University are beginning to focus the expertise and resources of botanic gardens, arboretums, and private collectors to improve offsite maintenance of wild plants.

Selecting Sites for Collecting

Studies of the geographic distribution of plants, the experiences of scientists and plant collectors, computer-based models, and re-

Photo credit M O'Grady

Collecting natural rubber (*Hevea*) in the Amazon forest. Natural rubber still has many industrial uses for which synthetic rubber cannot be employed.

Box 7-B.—The History of Plant Collection

Humans have collected plants and maintained them in collections for centuries (69,79,124). One of the earliest documented collecting expeditions was sent by Egyptian Pharaoh Hatshepsut in 1500 B.C. to obtain samples of the cedar tree. In 1492, Christopher Columbus returned to Europe with seeds of a new crop: corn. Later he took European wheat to the West Indies. As a result of such activities, the center of production of the world's agricultural crops today may be very distant from the places where the crops originated. Wheat, for example, is now a major U.S. crop for some European customers.

Botanic gardens were early repositories for the plants collected by explorers and grew in importance in the Middle Ages with the proliferation of pleasure gardens in Europe and the Middle East. During the colonial period of the 16th to 19th centuries, botanic gardens were established throughout the tropics. The palm, an important source of oil in Asia, was introduced in the early 19th century to Indonesia and Malaysia through botanic gardens in Java and Singapore. The Dutch East India Co. supplied trading ships with a variety of fresh fruits and vegetables from a botanic garden in its Capetown colony at the end of the 17th century.

The first botanic garden in the New World was established in 1766 by the British to acclimatize South Sea Island crops on St. Vincent (79). Captain William Bligh sought to introduce varieties of the starchy Tahitian staple, breadfruit, to British colonies. His first attempt ended in a well-known mutiny, but a later voyage in 1793 succeeded, and he brought six varieties of breadfruit to the botanic garden in St. Vincent. Though first rejected by the local population, breadfruit eventually became a regular part of Carribean diets. The garden at St. Vincent was instrumental in the introduction of many other important crops and spices to the Americas.

Medicine provided an important impetus to developing collections at many botanic gardens (79). These gardens were essential units within medical schools and similar institutions. The chemicals in many of these plants formed the basis for the pharmaceuticals industry, and some remain important sources for modern medicines (210).

Unlike many gardens of the 18th century, the Royal Botanic Gardens at Kew, England maintained a variety of plants, not just those of medical importance (79). Scientists at Kew developed a collection that today exceeds 50,000 species from throughout the world. Kew has been involved in the introduction of many agricultural crops important to Europe and North America, such as the tomato, potato, and rubber tree.

Introducing new crops became an official activity of the U.S. Government in 1819, when the Secretary of the Treasury enlisted the help of foreign diplomats and U.S. Navy personnel to collect plants from abroad. Prompted initially by the desire to introduce new plants and later by concern over loss of crop germplasm, the Federal Government instituted various national systems to collect, describe, maintain, evaluate, and distribute plant germplasm. These activities evolved to what is today the National Plant Germplasm System (115).

search on the origins of plants have enabled scientists to locate regions rich in diversity of crop species. The regions where most of the major crop species were originally domesticated and developed are known as centers of diversity, after a scheme first proposed by botanist N.I. Vavilov of the Soviet Union (45,57). At least 12 centers of diversity are now recognized (figure 7-1). Primitive varieties and related wild species that are able to survive in diverse habitats and resist a variety of crop-specific diseases can be located in these areas.

Some guidelines are available for collecting a genetically diverse sample *(12,47).* For instance, plants growing in areas of different soil, water, or light conditions may represent types that have genetic adaptations and could prove to be valuable sources of particular genetic traits. But guidelines for collecting seeds or

Photo credit Centro Internacional de la Papa (C/P)

Collecting wild potato species in South America, a center of potato diversity.

other propagation materials (cuttings, tubers, etc.) are only general and may be altered by specific conditions in the field. For example, the Central America and Mexico Coniferous Resources Cooperative (CAMCORE) has established guidelines for environmental and geographic factors to consider, depending on the number of trees to collect from, and the amount of seed to be collected (25,26). But if collectors encounter small populations of tree species, collection is made from any tree with seed (26). Thus, the guidelines for a collecting expedition depend heavily on the expertise and judgment of the individual collector.

Scientists and experienced collectors also provide helpful information on collecting sites. Several of the large so-called genetic stock collections of crop germplasm in the United States, such as the one for tomato at the University of California-Davis, are overseen by a few individuals with special interests in that crop. The knowledge these people have about origins and distribution of a crop is frequently the result of extensive observations and field collecting experiences.

Computer-based modeling is another potentially useful tool for predicting the location of sites appropriate for collecting. Data from a few key locations may provide information on the distribution of a particular crop trait, such as drought tolerance. A map can then be constructed by computer-based extrapolation of neighboring regions. Areas likely to contain plants with similar characteristics could be selected (2). However, this technology has its limitations. This kind of analysis requires precise data on latitude, longitude, and elevation for collected plants, for example—information that is not currently available in most crop databases (2). And because overall geographic information comes from satellite imagery, it can be prohibitively expensive (2). Political or other (e.g., geographic) restrictions on collecting in some areas may also make acquisition of plant samples difficult. Finally, data for the initial profile are obtained from sites chosen by statistical analysis, and plant distribution may not parallel these mathematically chosen sites. Refinements in existing databases, collection information, and artificial intelligence systems may someday allow such models to assist in collecting. However, it seems the importance of using existing data for such tasks has been overlooked (46).

Quarantine

After samples have been collected, complications may arise in transporting them. Movement of plants from one region to another always carries some risk that pests (nematodes, snails, insects, etc.) or pathogens (viruses, bacteria, or fungi) will also be transported (14,59,

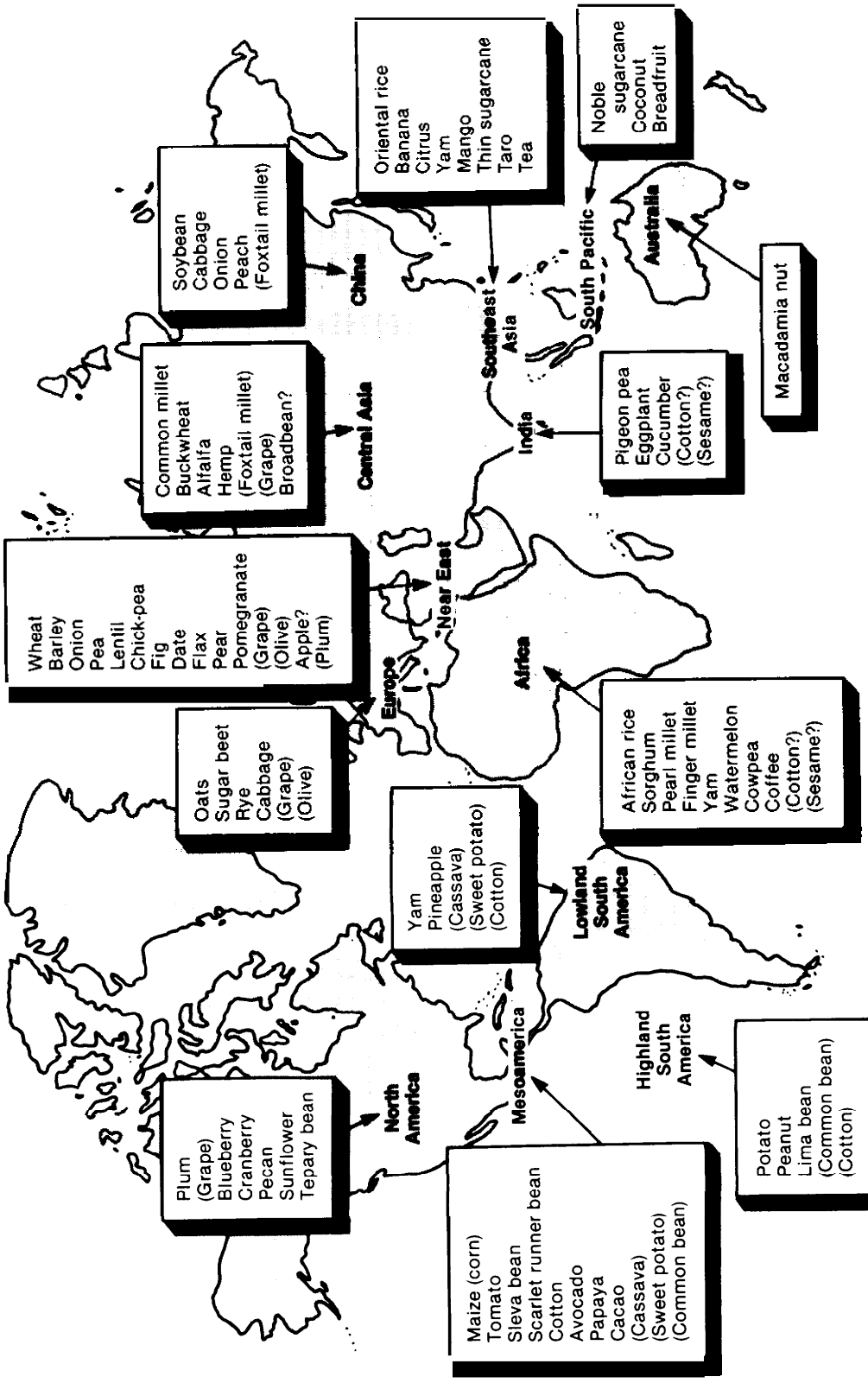


Figure 7-1.—Regions of the World Where Major Food Crops Were Domesticated

Crops that apparently originated in several different areas are shown in parentheses. A question mark after the name indicates doubt about the location of origin.

SOURCE: J.R. Harlan, "The Plants and Animals That Nourish Man," *Scientific American* 235(3):88-97, September 1986. All rights reserved.

60). Plants destined for offsite collections, particularly those from centers of diversity, can present particular quarantine concerns. These centers possess not only considerable crop diversity but also widely adapted crop pests and pathogens. An area where coffee and the disease coffee-rust coexist, for example, would be a likely place to obtain plants with rust resistance genes, but it could also have pathogens that have adapted to coffee plants (60).

The presence of most exotic pests can be determined by inspection or by treatment of plants upon entry, but some imported plants may be detained while tested for obscure pathogens. Such testing requires well-equipped laboratories and personnel as well as considerable time. This last constraint—5 or more years for the detection of certain viruses and virus-like organisms in woody plants—can profoundly affect importation of some plants (60).

Quarantine and plant Importation

Establishing quarantine policies and practices for a particular plant species depends on both knowledge of its risk of carrying pests or pathogens and availability of technologies to detect such pathogens (table 7-I). Most plant species, when imported according to regulations (e. g., clean and free of soil, and subject to inspection at an authorized port of entry), are considered unlikely to be carrying harmful organisms and thus to be of low risk (60).

Some agricultural crops, such as rice, sorghum, or sugarcane and their related wild species, require greater attention because they might contain pathogens not easily detected by current technologies.

Certain agricultural plants or plant parts used for vegetative propagation (e.g., sugarcane stems or potato tubers) represent the greatest risk to agriculture because they maybe infected with undetected pathogens (60). The U.S. Department of Agriculture (USDA) allows small quantities of these plants to be imported for scientific use only. Permits typically require that plants be grown under the supervision of a knowledgeable specialist and may require diagnostic testing for pathogens as well as specialized growing practices (60). Once plants have cleared safeguard restrictions, they may be distributed to the general public.

In the United States, some plants (e.g., apples, pears, and potatoes) are held at one of the Agricultural Research Service's Plant Protection and Quarantine facilities until they are considered free of any pests or pathogens *(60).* Plants in this group face the most constraints because the hazards associated with importing them are highest (60). These plants may be held for several years after their original importation.

In developing countries, plant quarantine systems may lack scientific expertise, facilities, or appropriate governmental infrastructures to support them (14). Therefore, they depend heavily on such regulatory constraints as import refusal, lengthy quarantine, or treatment for pests or pathogens, These restrictions can result in considerable delay in importation of plants to facilities in these areas.

Safeguards for Reducing Risk in Imported Germplasm

A number of actions and regulations, either at the place of origin or at the port of entry, reduce the risks associated with plant importation.

Inspection, certification, testing, or treatment of plants before export can reduce potential quarantine delays (60). Most plants require little more than inspection to move quickly through quarantine. In vitro plantlet cultures can, in some cases, be imported with fewer restrictions than the plants from which they originate, Such cultures, though, are not considered free of disease without diagnostic testing (60).

Upon entry, plants likely to contain pathogens can be tested with a variety of technologies (table 7-2) (59,60). However, procedures vary in reliability and in the resources they require. Indexing, which uses highly sensitive indicator plants, is the most reliable and wideIy used method, but it requires considerable greenhouse space to maintain the plants necessary for this test (60). Serologic methods that use an-

Table 7-1 .—Crops and Trees Commonly Prohibited Entry by Quarantine Regulations in 125 Countries

Crops and trees	Number of countries in which crops/genera are prohibited	Percentages of countries that name one or more pests or pathogens	Percentages of countries prohibiting: Plants only[a]	Plants and seeds	Seeds only
Forest crops.'					
Maple	14	43	100	0	0
Chestnut	34	23	76	24	0
Conifers[b]	27	26	100	0	0
Hawthorn	14	86	100	0	0
Walnut	21	48	100	0	0
Poplar	27	44	93	7	0
Oak	25	47	92	8	0
Willow	22	45	100	0	0
Ash	24	58	96	4	0
Elm	32	47	94	16	0
Fruit crops:					
Citrus	62	45	55	45	0
Coconut	28	32	29	64	7
Strawberry	20	55	65	35	0
Banana	39	39	54	46	0
Pome fruits[c]	37	68	85	15	0
Prunus (cherry, plum, etc)	37	68	85	15	0
Currant	16	38	69	31	0
Grapevine	41	41	90	10	0
Vegetable crops:					
Sweet potato	23	35	61	39	0
Potato	41	41	90	10	0
Other crops:					
Coffee	49	31	24	57	18
Cotton	52	23	25	61	14
Sunflower	15	40	20	80	0
Rubber	28	50	29	71	0
Tobacco	26	31	35	58	7
Oil palm	16	38	56	44	0
Rice	33	42	21	61	18
Rose	22	41	100	0	0
Cacao	43	42	19	79	2
Tea	20	45	45	55	0
Sugarcane	40	10	***63***	***37***	0

[a]Includes plants as well as any parts for vegetative propagation
[b]Specifically, the genera *Picea, Larix, Pinus,* and *Abies*
[c]Includes the genera *Chaenomeles, Cydonia, Malus,* and *Pyrus.*

SOURCE R.P Kahn, "Technologies To Maintain Biological Diversity" Assessment of Plant Quarantine Practices," OTA commissioned paper, 1985.

tibodies to pathogens provide rapid results but may not detect all forms of a particular pathogen **(60),** Molecular techniques to detect the genetic material of pathogens are available,but these may require better-equipped laboratories and greater expertise than is available in many quarantine programs, Identifying the presence of a pathogen can thus be difficult because a negative result maybe due to inadequate technology. It is essential, therefore, that the limits of any technology be understood. Basic research on the biology of pathogenic organisms and the technologies used to detect them is needed to improvetesting procedures, develop them for other pests and pathogens, and understand the limits.

STORING SAMPLES

Storage technologies aim to preserve an adequate amount of plant germplasm, sustain its viability, and preserve its original genetic constitution (81). Plants can be maintained offsite in a numberofforms and with a number of technologies (table 7-3). They may be maintained

Table 7“2.—Technologies and Practices To Detect Pests and Diseases of Quarantine Significance and the Pathogens To Which They Are Most Frequently Applied

Technology/practice: Description	Range[a]
Physical examination: Physical manifestations of disease-producing agents.	A
Seed health testing: Germinating seed in culture conditions that allow growth of fungi or bacteria. Microscopic examination of seed for pathogens.	B,F
Grow-out testing: Growing plants under controlled conditions until diseases are no longer detected	B, F,V,O
/ndexing: Attempted transfer of pathogens from a plant under examination to another species that is highly sensitive to infection by them. Can involve transfer by mechanical abrasion with extracts or by grafting	B,F,V
Electron microscopy: Examination of extracts or tissues for the presence of pathogens or their spores	B,V
Inclusion bodies: Light microscopic examination of tissues for structures characteristic of pathogen infection.	B,F,V
Serologic testing: An array of procedures utilizing the ability of test animals to produce antibodies that are highly specific for a particular pathogen. Important variations include enzyme-linked immunosorbent assay, radioimmunosorbent assay, and immunosorbent electron microscopy	B,V,O
Polyactylamide gel electrophoresis: Detects ribonucleic acid (RNA) of pathogens in small amounts of tissue	B,V,O
Nucleic acid hybridization: *New* technology that uses recombinant DNA procedures to locate the genetic material of a pathogen in DNA extracted from tissue samples.	B,V,O

aA = Most pests and pathogens, B = bacteria; F = fungi, V = wruses, O = others (0 g , ffIYCOPlaSmaS, vIrolds)

SOURCE Based on data from R P Kahn, “Technologies To Ma!ntaln Biological DIvers!ty Assessment of Plant Quarantine Practices, ” OTA commissioned paper, 1985

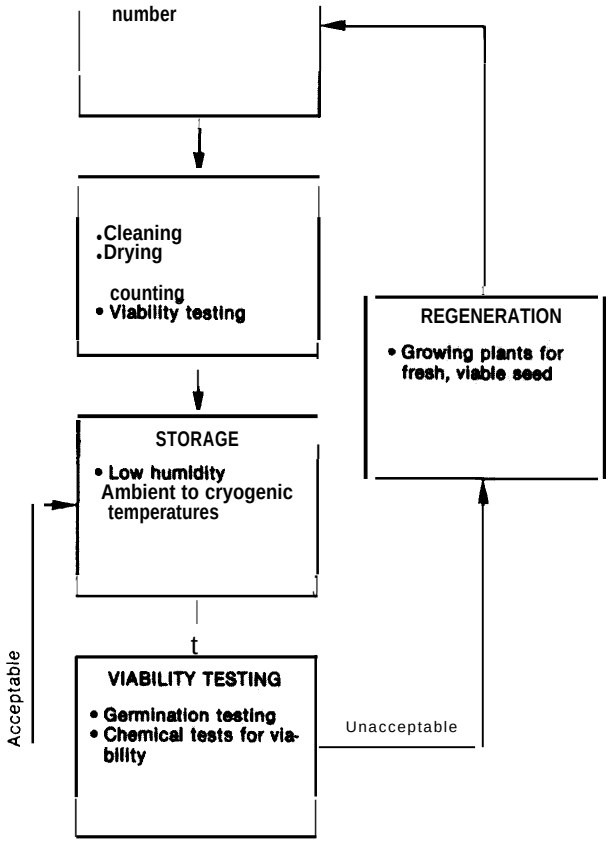
Table 7-3.—Storage Technologies for Germplasm of Different Plants

Plant group	Storage form[b]	Storage technology: Field collections	Storage technology: *In vitro* culture	Storage technology: cool temperature	Storage technology: Liquid nit rogen	Collection[c]
Cereals and grain legumes (wheat, corn, barley, rice, soybean)	seeds			x	R	B,A
Forage legumes and grasses	seeds			x	R	B,A
(alfalfa, orchardgrass, bromegrass, clover)	plants	x	X,R	x		A
Vegetables	seeds			x	R	B,A
(tomato, bean, onion, carrot, lettuce)	plants	x	R	R	R	A
Forest trees	seeds			x	R	B,A
(pines, firs, hardwoods)	plants	x	R	R		B,A
Roots and tubers	seeds			x		B,A
(potato, sweet potato, tropical yam, aroids)	plants	x	X,R	R	R	A
Temperate fruit and nuts	seeds			x	R	B(?),A
(apple, grape, peach, strawberry, raspberry)	plants	x	X,R	R	R	A
Tropical fruit and nuts	seeds			x	R	A
(avocado, banana, date, citrus, papaya, cashew)	plants	x	R	R	R	A
Ornamental	seeds			x	R	A
(carnation, zinnia, lilac, rhododendron)	plants	x	X,R			A
Oilseeds	seeds			x	R	B,A
(soybean, sunflower, peanut, oilpalm, rape)	plants	x	R	R		A
New *crops*	seeds			x	R	B,A
(jojoba, amaranth, guayule)	plants	x	X,R			A

ax ~ currently i„use, R = under research and development.
bRefers to source of materlafs for storage (e,g , plants are the source of materlais for initiating tissue Cultures
CB ~ base Collections available, A = active COIIOCtIOnS available

SOURCE Adapted from L Towill, E Roos, and P C Stanwood, “Plant Germplasm Storage Technologies,” OTA comm!ssloned paper, 1985

Figure 702.— Maintenance Process of a Plant Seed Bank



SOURCE: Office of Technology Assessment, 1986.

as seeds, in fields, or in greenhouse collections. Pollen storage and in vitro plantlet cultures may supplement storage of many of these species. Finally, cryogenic storage (in liquid nitrogen) and emerging DNA technologies may hold potential for improving maintenance of plants.

Conventional Seed Storage

Most agricultural crops held by the National Plant Germplasm System (NPGS), international centers, national programs, and private collections are maintained as seeds. The process of conventional seed storage can be divided into several steps: registration, processing, storage, viability testing, and regeneration (figure 7-2).

Registration

When seeds arrive at a storage facility, passport information must be recorded and a number assigned to facilitate recordkeeping. Passport data may include information on the origin of the sample, its source (if acquired from another facility), and any pertinent physiological details that would aid storage. Data of interest to potential users, such as disease resistance, also may be included.

The information accumulated may reflect the focus of a particular collection. The Royal Botanic Gardens at Kew, England, requires details on the location and habitat in which a wild plant species was collected, an estimate of the total number of plants represented by the sample, a taxonomic classification, and the location of a reference herbarium specimen. The more detailed this preliminary information is, the more useful the accession is for crop development or conservation.

Collections may receive the accessions of another collection, thus duplicating materials. Although such duplication does provide security against loss, the number of accessions held by all collections does not reflect duplicates. In barley, for example, the total of more than 280,000 accessions in storage is considerably greater than the estimated 50,000 distinct accessions worldwide (70).

Processing

Once registered, other data such as estimates of the number of seeds received, viability in terms of percentage of germination, and taxonomic identification must be obtained. In addition, seeds may require preparation, like cleaning and drying for storage.

Seeds are tested for germinating ability to determine if the sample is of high viability or whether it must be planted to produce fresh seed before storing (29,30,31,43).

To reduce moisture in seeds, procedures using chemical desiccants have been developed and are widely applied (111). Facilities with large amounts of seeds to process, such as the U.S. Plant Introduction Stations or the National

Seed Storage Laboratory (NSSL), use dehumidified rooms to reduce moisture.

Storage

Four factors affect seed storage: 1) moisture content, 2) storage temperature, 3) storage atmosphere, and 4) genetic composition of the sample (4,87,88,89), Reduced moisture content is considered the most crucial to maintaining viability. In general, each l-percent decrease in seed moisture between the 5- and 14-percent range will double the lifespan of a seed sample. Reduction of storage temperature also increases seed longevity. A 50 decrease in temperature between 0° and 50° C doubles longevity (89). Control of storage atmosphere generally does not provide significant advantages over manipulation of moisture and temperature, particularly when the latter is below freezing. Genetics relate to differences between individual accessions or between individuals in a mixed sample and cannot be altered to increase longevity.

Viability Testing

Seeds must be tested periodically for viability. This information helps determine when an accession needs to be grown-out to produce a fresh sample of seeds. The most obvious test is to germinate a portion of the seeds to estimate the viable percentage.

Viability testing involves placing seeds in appropriate conditions (damp blotter paper, agar medium, etc.] and counting the number of seeds that germinate over a period of time. However, if seeds are dormant, obtaining viability estimates can be difficult. Citrus species, for example, were thought to have died when prepared for conventional storage but were shown instead to be dormant (82). Heating, cooling, lighting, and treatments (e. g., removal or cracking of the seed coat) may be required to overcome dormancy in some species.

Typically, 200 to 400 seeds are required for viability testing (43). However, a sequential approach reduces the number needed for testing. Forty seeds can be tested to determine whether the accession should be regenerated, stored, or whether another 40 seeds are needed (30,42). But a small sample of seeds may need to undergo numerous tests before an answer is reached, which may take longer than testing a single large sample.

Other tests–involving dyes, physiological tests, or biochemical assays—have been developed to determine seed viability (89). The validity of such tests relies on the ability to demonstrate a correlation with actual germination rates. These tests can be useful to determine if dormancy or inappropriate storage conditions are producing misleading results (30). Some, such as the tetrazolium dye test for a range of seeds, or X-ray contrast with heavy metals in tree seeds, have been widely used (30,89). others, such as enzyme tests, provide information useful for the study of seed physiology but are more expensive and difficult to perform than standard germination tests.

Preserving the genetic variability in seed accessions is a major concern in offsite collections, Many accessions, particularly those of primitive landraces and wild species, are genetically diverse populations and display considerable genetic variation between individuals in a sample. Genetic differences in storage lifespan can mean that the genetics of a population could be altered by decline in viability (86,87,88,108).

Although seeds generally should be regenerated when germination drops 15 percent, practical considerations of labor, space, and time can delay this step (32,43,83,108), One recent report stated that NSSL does not regenerate samples until viability has dropped 40 percent (113). This practice, however, may be based more on lack of resources than on scientific considerations.

Regeneration

Variations in growth requirements for species and even for varieties within a species complicate the growing-out of seed. In beans, for example, different accessions may *require* different conditions, e.g., daylength, for growing out. Thus, both subtropical and temperate sites must be used to grow-out a range of bean

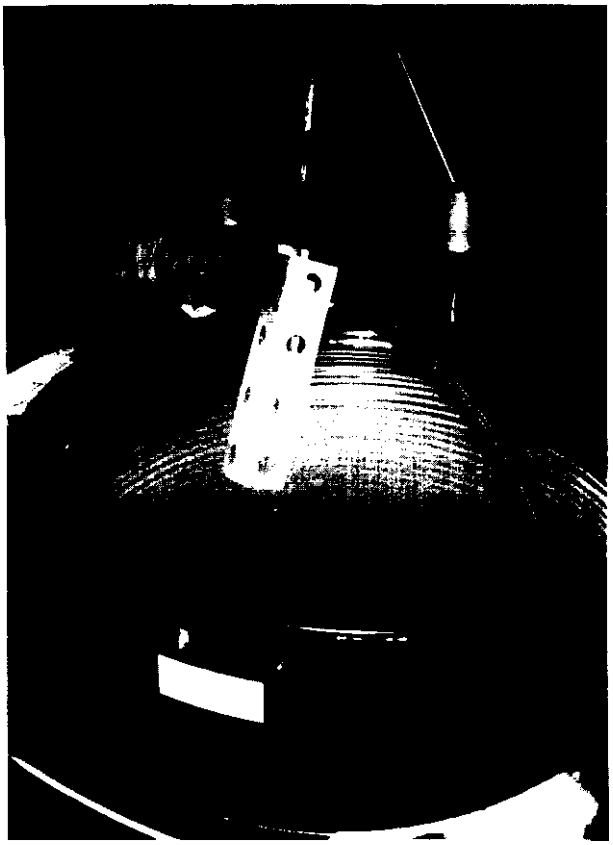
Photo credit: OTA staff

Conventional seed storage. Seeds are stored in airtight containers (top photo), then placed in drawers (bottom left) in refrigerated rooms (bottom right). Many storage facilities increasingly use laminated foil envelopes instead of cans.

varieties, Other factors such as control of pollination can also be important for regenerating certain crops (95,108). Wind-pollinated accessions can readily cross with others, and thus, individual accessions must be grown in widely separated fields to ensure that they are not genetically mixed.

Genetic loss by natural selection during grow-out may be undetected in regenerated seed. At NSSL, the designated grower is responsible for ensuring that the sample returned is from plants grown under conditions that would minimize genetic loss. No testing beyond visual examination and a viability test of the returned sample is conducted.

Grow-outs are expensive in terms of facilities and personnel, and they subject stored materials to damage from pests, pathogens, and environmental conditions, which may reduce genetic diversity in an accession. But the most effective and least expensive way to maintain diversity is to reduce the frequency of regeneration through technologies that extend storage.



Cryogenic Storage of Seeds

A critical factor in cryogenic storage is the amount of water in the tissue to be frozen. Most orthodox seeds can be easily stored at cryogenic temperatures because their water content is low enough to avoid damage associated with freezing (99,100,102,108),

Cryogenic technologies may be able to extend the storage life of orthodox seeds to more than a century, which would greatly reduce the need for viability testing and regeneration (100,102, 121,122).

However, limitations on cryogenic storage exist for some species depending on a plant's seed coat, oil or moisture content, and seed size (108). Some plants, such as plums and coffee, have orthodox seeds that tolerate low moisture levels but are sensitive to cooling below –400 C (100). If cooled or warmed incorrectly, many seeds can crack (100). Seeds as big as cotton seeds (about eight seeds per gram) are appropriate for cryogenic storage (99,100,102). Larger seeds, such as beans, can also be frozen, but increased costs, due in part to the greater amount of space required, may reduce or eliminate potential cost-savings over mechanical refrigeration (102).

This method could hold considerable cost advantages with regard to operating a seed bank and regenerating seed (table 7-4) (102). Cryogenic storage facilities will cost about the same to establish, but operation over time would be cheaper, in part due to reduced need for viability testing and grow-out. Investment in a facility to produce liquid nitrogen might be necessary in some areas, but the operational savings in the seed bank could allow recovery of costs in 6 to 14 years (99).

Major obstacles to this technology are the lack of appropriate facilities and scientific exper-

Table 7-4.—Estimated Costs[a] of Conventional and Cryogenic Storage

Source	Storage for 100 years	
	Conventional[b]	Cryogenic
Storage: Includes equipment, supplies, and replacement of equipment	$ 5.30	$5.00
Operations: Includes utilities, equipment maintenance, liquid nitrogen coolant, and monitoring of viability (every 5 years for mechanical; every 50 years for cryogenic	60.00	11.80
Seed *replacement:* From regenerating when viability or sample size declines (four times for mechanical; one time for cryogenic)	100.00	25.00
Total 100-year cost	$165.30	$41.80
Average yearly cost	$ 1.65	$0.42

%osts for accession of **onion** (a species that survives poorly under conventional **storage). Savings for other crop species-particularly those with large seeds—may be less dramatic or nonexistent,**
bAss$_{ume}$s storage conditions of – 18° C and seed moisture of 4 to 7 Percent, under which **storage life of onion seed is approximately** 25 years.

SOURCE: P.C. Stanwood and L.N. Bass, "Seed Germplasm Preservation Using Liquid Nitrogen," *Seed Science* **and** *Technology 9:423-437,* **1981.**

tise at many locations, particularly in developing countries, and the lack of scientific data on genetic stability of seeds stored cryogenically. Certainly the capacity to use cryogenic technologies should be part of any newly constructed facility for seed storage.

Field Maintenanence and Controlled Environmomts

Accessions may also be stored as vegetative plants in field collections or controlled environments e.g., greenhouses (108). This approach may be necessitated by physiological restrictions on storing seed, by the need to preserve particular combinations of characters or by inabilities to obtain satisfactory seed samples (81,108). Field collections can preserve the genetic diversity of many aquatic plants; tropical species (e.g., coconut, cacao, mango, or rubber trees); tropical forest trees; and some temperate trees (e.g., oaks) —which all have recalcitrant seeds (28,42,63,64,84).

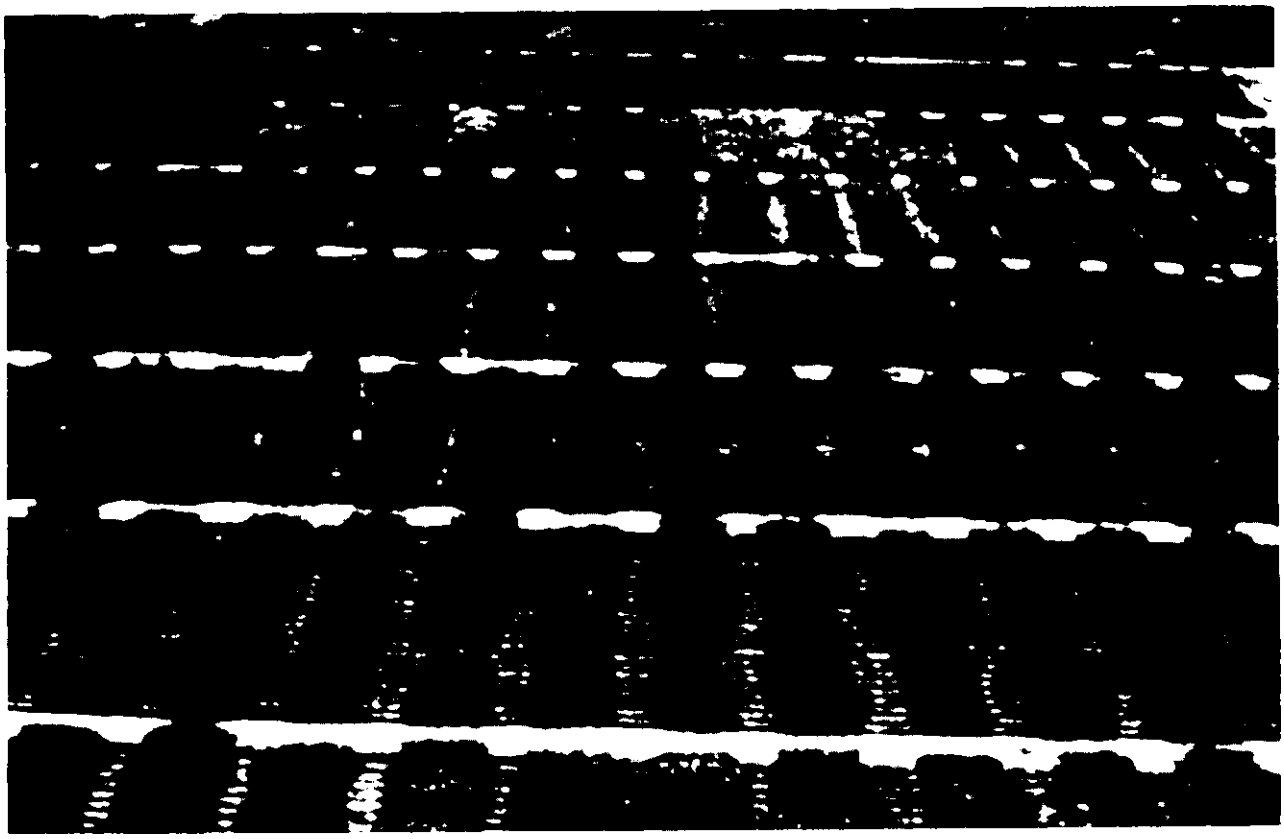
Botanic gardens and arboretums maintain diverse field collections, though many institutes focus on a narrow taxonomic group, as mentioned earlier. Arboretums conserve limited samples of tree and shrub species with very small natural gene pools that are under pressure of destruction, or plants with distinctive characteristics (34,69,80). In the United States, establishing a network among botanic gardens and arboretums, facilitated by the newly formed Center for Plant Conservation at the Arnold Arboretum at Harvard University, could allow a division of labor and sharing of expertise that would enable more species and more genetic diversity within species to be maintained (34, 106).

Trees in field collections, however, may have been selected for economically important traits and thus may only represent a narrow range of the total diversity available for a species. CAMCORE, for example, collects seeds only from coniferous trees with commercially valuable trunk characteristics (i.e., tall and straight) (25). Trees in field collections, nonetheless, can be useful sources of seeds for restoration and reforestation projects (8).

Many clonally propagated crops are maintained in field collections. Clonally propagated crops include fruit and nut species; many ornamental, such as roses; and some root and tuber crops important to developing countries (e.g., sweet potato, cassava, and tare). Seeds may be available for many varieties. However, most of these crops are genetically heterogeneous, and clones grown from their seeds may not retain the particular qualities of the parent plants (e.g., the seeds of a Macintosh apple do not produce Macintosh apple trees, but rather a range of trees that result from recombination of the genes in the Macintosh apple). The Centro International de la Papa (CIP) in Peru, for instance, maintains an active field collection of potato landraces but also has abase collection of seeds from these accessions (50).

Pollen Storage

Pollen is not a conventional form of germplasm storage, but information is available on preserving pollen for breeding purposes for



many species, particularly for crossing materials that flower at different times (107,108). A population of pollen grains collected from genetically different individuals would contain the nuclear genes; cytoplasmic (nonnuclear) genetic factors would not be transmitted, however, because these are not inherited through the pollen (108).

Pollen can be separated into types that are tolerant or intolerant of drying (81,107,108). Tolerant types store best when dried and maintained at low temperatures, much like orthodox seeds. But the pollen of many species does not survive low moisture or freezing temperatures. Some intolerant types, notably maize, have been successfully preserved in liquid nitrogen, but data on success are sparse (3,107, 108).

Considerable information is still needed on stability and longevity of storing pollen, however, before its use in storage will be possible (108). Pollen is undesirable as the sole propagule for base collection storage because whole plants cannot generally be obtained from it (81,108). In addition, pollen storage does not circumvent potential plant health problems because some pathogens are pollen-borne,

Biotechnology

Biotechnology provides additional opportunities to improve offsite maintenance of plants. Of particular relevance are in vitro cultures of plants that are now maintained in field collections. And developments in genetic engineering may make the storage of isolated DNA practical in the future.

In Vitro Culture

In vitro cultures of plants have been advocated for a variety of species, especially those that are clonally propagated (20,53,56). Although this technology can be adapted to many species (16,33,93), it is generally unnecessary for those that have orthodox seeds. However, the methods may be necessary if there is a need to maintain specific genetic types, if seed progeny are highly variable, if plants have long juvenile stages (e.g., many tree species), or if seeds are lacking (e.g., clonal crops such as banana, tare, and sugarcane) (20).

In vitro maintenance is defined as the growing of cells, tissues, organs, or plantlets in glass or plastic vessels under sterile conditions (108). when plants originate from intact isolated meristems, the cultures may be free of pathogens (108). The media for growing in vitro cultures may vary among species and among individuals within a species. By altering the balance of nutrients and growth regulators in the media, in vitro cultures can be made to develop unorganized growth (termed callus), produce multiple shoots or plantlets, form structures similar to the embryo in a seed, or develop roots to enable transfer to field conditions. Not all plants, however, are amenable to growth or manipulation by in vitro culture (108).

One aspect of in vitro technology of particular concern for plant germplasm conservation is the occurrence of genetic modification (somaclonal variation) in plants derived from callus cultures (67,90). Such variation is considered useful in the development of new varietal characteristics but is unacceptable when preservation of specific genotypes is the objective. Although it is known that certain types of cultures and conditions, such as callus cultures, can produce higher frequencies of somaclonal variation, the cellular processes that produce them are not well understood (90,120). Furthermore, growing cultured plants to maturity remains the only satisfactory way to examine the consequences of such changes. Consequently, each method of culture must be carefully evaluated before it is applied. For germplasm preservation, in vitro plants directly derived from buds or shoot-tips are considered most suitable.

Photo credit: International Board for Plant Genetic Resources

In vitro culture could become an important method of long-term maintenance for plants with seeds that cannot be stored under dry, cold conditions and for plants that can only be maintained in field collections.

No in vitro long-term base collections of agricultural crops exist at present, although some active collections are being developed: potato at CIP, cassava at the International Center for Tropical Agriculture (CIAT) in Colombia, and yam and sweet potato at the International Institute for Tropical Agriculture in Nigeria (122, 123). The NPGS Clonal Repositories are investigating in vitro cultures as backup to field collections of some crops (108).

In vitro cultures can be stored under normal growth conditions, in reduced temperatures or

in a medium that inhibits growth (53,56,119, 120,122), Cultures can thus be maintained for weeks to months without subculture (i.e., transfer to fresh medium). However, all treatments that retard growth put additional stress on the culture, which may increase the potential for somaclonal variants.

In vitro culture techniques could be important for the long-term maintenance of plants with recalcitrant seeds. One recent proposal has been to excise the embryo from the seed and store it under cryogenic conditions. The embryo could be thawed and then grown and multiplied in vitro (40). Research in cooperation with the Royal Botanic Gardens at Kew, England, has demonstrated the feasibility of this procedure for two tree species (Araucaria husteinii and Quercus robur). Further research is needed to apply it to other plants with recalcitrant seeds (4o).

Cryogenic storage may help avoid the stresses of continuous in vitro culture (49,62,90,121,122, 123), Considerably greater attention would be needed in preparation, freezing, storing, thawing, and subsequent culturing than is the case for orthodox seeds. Although some generalizations can be made, methods acceptable to one species or variety may not be satisfactory for others. However, research has demonstrated that *in* vitro-cultured shoot-tips from some herbaceous plants (e.g., potato, carnation, and cassava); berries (e. g., strawberry, raspberry, and blueberry); and buds of some woody species (e.g., apple) can survive cryogenic storage (108).

Many questions remain before cryogenic storage of in vitro cultures is widely applied. Among these is whether a single procedure can be developed that works well for an array of plants. Further, it is not yet understood how the process of freezing and thawing affects regeneration of cultures or their genetic constitution (108,121). Additional investigation for individual crops is needed, and current technologies have not yet been adapted for handling the large numbers of specimens that might be expected in an offsite facility.

DNA Storage

Future storage technologies may include, as a supplemental strategy, the preservation of the isolated genetic information (DNA and RNA) of plants. Existing technologies can locate, excise, and reinsert genes. In some cases, these genes retain nearly normal function (75,108). A much better understanding of gene structure, function, and regulation is needed, however, before isolated DNA can be used for germplasm storage (76,108).

Management of Stored Materials

Offsite collections of plants must be well managed to guard against loss of materials and to use financial and technological resources most efficiently. Some duplication between collections can prevent catastrophic loss, but excessive redundancy can waste resources. Disease organisms that might be brought into a collection by new accessions need to be managed. Finally, information on the accessions must be easily available both to managers and users.

Duplication of Collections

Duplication of collections provides the best insurance against natural catastrophes, pests, diseases, mechanical failures, or abandonment (81,108), CIP protects its collection of landrace potatoes with field plantings at other locations, with seed storage, and with *in vitro* culture (50). At the NPGS Clonal Repositories (see ch, 9), greenhouse collections back-up field-maintained collections of fruit and nut species. Duplication is equally critical for seed banks, where malfunctioning of a mechanical compressor can result in loss of cooling.

Plants in an offsite collection also can be lost if institutional priorities change, or if the person responsible for the species or collection leaves (108). The situation is particularly critical for older varieties of fruits, berries, and vegetables that may be held only by private individuals or groups (112). For wild species, too, a large collection is frequently the result of the

interest of one person or a few individuals. when these efforts cease, a valuable collection can rapidly deteriorate. Information on the focus and extent of various collections can aid coordination of duplication and minimize the potential for loss (34,112).

Assessing Diversity in a Collection

The diversity of a collection can be assessed by collecting morphologic, biochemical, or phytochemical information, frequently called characterization data.

Most characterization data can help distinguish one accession from another but not assess potentially useful traits. This is particularly true for assays of proteins or DNA, which give little indication of such traits as crop yield, disease, or stress resistance.

Morphological Assessment.—Assessing the morphology of an accession is the first step to developing accurate characterization, Morphological information for wild species is important for taxonomic identification and forms the essential baseline from which all other data are related (68). The information on agricultural crops can be used to distinguish individual accessions, as well as identify them taxonomically (114). However, data are on gross appearance and do not reflect the full genetic composition of an accession.

Care must be taken to ensure reliable results when plants are grown-out for morphological assessment (10). Spacing of plants must be adequate to ensure that results do not reflect overcrowding, for example. Samples thought to be duplicates are frequently grown side by side for comparison (13). Biological factors, such as the potential for cross-pollination among accessions, must be taken into consideration.

The major constraints to assessing morphology are adequate space, funds, and trained personnel. Though not technically difficult, such assessments require attention to possible environmental effects and may take a significant amount of time to perform, analyze, and record,

Biochemical Analysis.—Analysis of proteins or DNA using electrophoretic techniques is another way to assess diversity (94). Isoenzymes, the protein products of individual genes, can change in number or chemical structure when the genes for them are altered, and these changes can be detected by an electrophoretic assay, Examination of DNA can allow comparison of the entire genetic composition of accessions.

Isoenzyme analysis on either starch or polyacrylamide gels has probably been the most popular technique for assessing genetic diversity. Surveys of isoenzyme polymorphism have been performed for maize, wheat, tomato, pea, and barley (94,114). In addition, surveys have been done on hundreds of other cultivars and wild species (94,104, 105,114).

A potential application of this technology is the development of isoenzyme “fingerprints” to permit reliable identification of specific plant varieties to certify breeding materials, to isolate genetically similar cultivars, or to monitor otherwise undetected genetic changes in accessions. Electrophoretic analysis has been used to detect duplication in some offsite collections, such as the CIP collection of potato germplasm (50) and is increasing in application at NPGS facilities (19). However, since the data are generally restricted to a few biochemical characteristics and do not reflect performance data or the full genetic composition of an accession, such analysis has been considered risky (39).

Two-dimensional electrophoresis is used to separate complex protein mixtures such as those found in seed or leaf extracts so that several hundred proteins can be distinguished in a single gel (9,17,114). The results can be difficult to reproduce, however. The technique requires specialized equipment and may be too lengthy for routine use because only one sample can be evaluated at a time. Managers of offsite collections are unlikely to have the time, expertise, or resources to use this technique routinely.

Restriction fragment length polymorphisms (RFLPs) have been used to directly examine

DNA. DNA is "cut" enzymatically (using restriction endonuclease enzymes) into pieces or restriction fragments that can be separated on electrophoretic gels. Because RFLPs represent the whole genetic composition of the sample, comparing individual analyses within a sample or among accessions would indicate the variability that exists. The techniques, however, are expensive and require technicaI expertise to execute and interpret. Thus, use of RFLPs appears limited at present to appropriately equipped laboratories (114). RFLPs have been useful in developing detailed genetic maps for use in breeding programs (48) but are not routinely applied to characterization of germplasm.

Phytochemical Analysis.—Phytochernical analysis deals with the distribution and chemistry of organic compounds synthesized by plants *(114).*

Analysis involves three general processes: extraction, isolation, and identification (44,114). plant materials are homogenized in aqueous alcohol, then purified by evaporation of the alcohol and chemical partitioning to remove contaminating substances and isolate the chemicals of interest (114). Chemicals can then be identified by chromatographic or spectroscopic techniques (114).

During the past 10 years, the major technological advance in separation and purification of organic chemical mixtures has been the development of high-performance liquid chromatography (H PLC). HPLC is more rapid than other chromatographic procedures and can isolate a range of plant chemicals. Developing appropriate HPLC procedures, however, requires considerable investment of funds and time. Some facilities of NPGS, however, are using techniques such as HPLC to help characterize germplasm (19).

Recent advances in microcomputers have provided sophisticated, low-cost spectrophotometers that can identify plant chemicals (114). Other techniques, such as nuclear magnetic resonance spectroscopy and mass spectrometry, also can determine chemical structure but they require considerable technical expertise and expensive instrumentation. These technologies have been used extensively, however, by scientists studying the taxonomy and systematic of plants (114) and by university and industry scientists interested in developing potential uses for wild plants in medicine and industry.

Controlling Pests and Pathogens in Collections

Managing stored samples requires efforts to ensure that seeds or plants are not lost to pests or pathogens. Because a collection may distribute seeds to other regions, precautions must be taken to reduce the possibility of sending pests or pathogens as well (61).

Stored seed can be severely damaged by rodents, insects, or fungus (58). With rodents, the major damage is not from consumption but rather from the pests scattering and mixing up different accessions (58). Many insect, fungal, and bacterial contaminants can be controlled with the use of chemical fumigants, although such treatments might also harm the seeds (58, 59,81). Sanitary storage facilities that obviate the need for such treatment are therefore preferable (81). when dried seeds are kept at subfreezing temperatures, the potential risk is minimal (58,101),

The risk of disseminating pathogens is considerably greater for crops maintained through clonal propagation (59,61). Some facilities with a specific focus may have greater expertise with a crop and its diseases than a national quarantine facility concerned with all potential introductions. Cooperation between scientists and quarantine officials can improve control of pathogens and aid technology development.

Imformation Management

Offsite collections are repositories not only of germplasm but also of information, This information can aid collection management, can provide more efficient access to specific accessions, and might help develop collection strategies.

The current focus has been on standardization of terminology in order to facilitate exchange between collections and to provide

more consistent information to users (117). Development of uniform crop descriptors that include information about the storage history of an accession as well as data on the original collector, collection site, vegetative and reproductive characteristics, disease or pest susceptibility, and biochemical characteristics (e.g., isoenzyme profiles) is important for consistent and accurate information (7,98,117). This task for NPGS has been assigned to crop advisory committees (see ch. 9).

Several data storage and retrieval methods are now used (65). A collection of only a few hundred accessions might use file cards or books. As the collection grows, a computer-based system may be more appropriate. Large collections, such as the Royal Botanic Gardens in England, have developed systems adapted to their specific needs (97). The nature of the data in computer-based information management systems depends on whether the materials being stored are agricultural crops (1,125) or wild species (34,97).

The Germplasm Resources Information Network (GRIN) of NPGS is an example of a large information system designed to coordinate data from multiple collections in the United States. GRIN, once fully established, is expected to provide information on all accessions held by NPGS. Although the capacity of the system is more than adequate, entering information is, after several years, still in the preliminary stages. OTA has found GRIN praised by managers of NPGS facilities for its recordkeeping operations but criticized by potential users because detailed information is unavailable on individual accessions and obtaining results of searches can take considerable time. GRIN at present does not collect information on privately held collections of agricultural plants, such as those coordinated by the Seed Savers Exchange or the North American Fruit Explorers, nor does it hold information on wild species.

USING PLANTS STORED OFFSITE

Collections are used for crop development as well as conservation. Plant breeders and scientists who may depend on the genetic diversity in such collections require specific information about accessions to select appropriate plants. Genes in selected accessions are incorporated into improved crop varieties using traditional plant breeding practices. In addition, biotechnology may provide methods that could enable development of improved crop varieties or more efficient use of genes in plants.

Evaluation

Evaluation of plant germplasm involves the examination of accessions for the presence and quality of particular traits that may be of use to crop breeders.

In general, evaluation examines traits that may be genetically quantitative (i.e., controlled by many genes) and subject to environmental influence, such as drought tolerance or earliness of maturity in a fruiting crop. This assessment can be complicated in a genetically variable accession because all individuals may not express the trait equally *(81).* Further, changes in conditions (e.g., appearance of a new disease) can require further evaluation for new traits. In addition, new accessions must be evaluated. Thus, evaluation may be considered a never-ending task (81).

Evaluations vary according to the species or trait being examined and may be both lengthy and complex (37). A test for yield potential, for example, would require different growing conditions than a test for genes that enable plants to grow in acid soils. And sufficient space to grow plants to maturity is needed, along with trained personnel to design the tests and to analyze results (81). The time required too can be considerable. Testing the wheat held by the U.S. National Small Grains Collection for resistance to stem and leaf rust, for example, is expected to require more than 10 years (96). Evaluation

of some traits may require repeating tests over several years and in different regions (81).

Evaluation has been perceived as a serious deficiency in the overall effort to maintain crop germplasm (23,37,78,108,117). Insufficient information has meant accessions have been underused. However, the situation has been improving (81,108). International Agricultural Research Centers have evaluated many of their accessions for important traits, chiefly disease and pest resistance, yield, and quality factors (13,50,108). In the United States, the four regional plant introduction stations (see ch. 9) have included examination for several agriculturally important traits in their preliminary characterizations. This is not, however, sufficient to meet all the needs of users, and more extensive efforts are necessary (108).

Although the usefulness of a collection may depend on its evaluations, questions remain as to who has responsibility for this task. Collection managers might be able to gather morphological data, but they may not be able to perform the lengthy and detailed trials needed to evaluate traits. Further, it has been argued that such evaluations are best done under the conditions in which they will be used because expression may be altered by environmental differences (36). It would seem, therefore, that evaluation trials for specific traits are best performed by the breeders who require those traits. Duplicating efforts could be minimized by putting results into centralized database systems—a proposed function of the GRIN system in the United States,

Traditional Breeding

Traditional breeding typically involves identifying particular genes or characteristics and incorporating them into existing varieties. Crop development through breeding, a major contributor to modern gains in agricultural production, is a time-consuming process: It may take 10 to 15 years to develop a single new variety (box *7-C).*

Traditional breeding has provided as much as 60 percent of the production increases of many agricultural crops (22,24,35,77,124). Nevertheless, the process must continue in order to sustain agricultural yields—pests and diseases adapt to new varieties, and the needs of growers and consumers constantly change (77).

Traditional breeding involves several basic steps: 1) locate a genetically stable trait (e.g., yield, pest resistance, or stress tolerance); 2) isolate plants with the most desired expression of the trait; 3) breed genes into breeding lines of plants similar to those that will be improved to provide more usable material; and 4) cross these plants with other breeding lines to produce plants from which improved crop varieties can be selected (41,81).

The third step, called developmental breeding, is important because the desired trait may be located in a wild species or variety that is difficult to cross with domesticated ones. This is the case, for example, with genetic resistance to some 27 serious diseases of the tomato (81). wild species or landraces may have different growth requirements that make crossing them with other varieties difficult. Developmental breeding overcomes such differences but may require growing plants at multiple locations. Incorporation of genes from over 500 exotic sorghums, for example, required growth in two locations because the exotics required shorter days to flower than commercial U.S. varieties. A cooperative effort, therefore, was established between the Texas Agricultural Experiment Station and the USDA Federal Station in Mayaguez, Puerto Rico, to perform the crosses and test the progeny (48,81).

The major constraint to traditional breeding is its dependence on the sexual process of plants. Multiple crossings and testing of offspring may take years. Molecular biological techniques to locate and map genes in plants may greatly shorten the time needed for breeding improved varieties (6).

Biotechnological Improvement

Biotechnology provides greater precision and speed in the manipulation of genes by avoiding the sexual reproductive process (24,41,75). Three general areas have potential impact on the use of stored plant diversity: 1) somaclonal

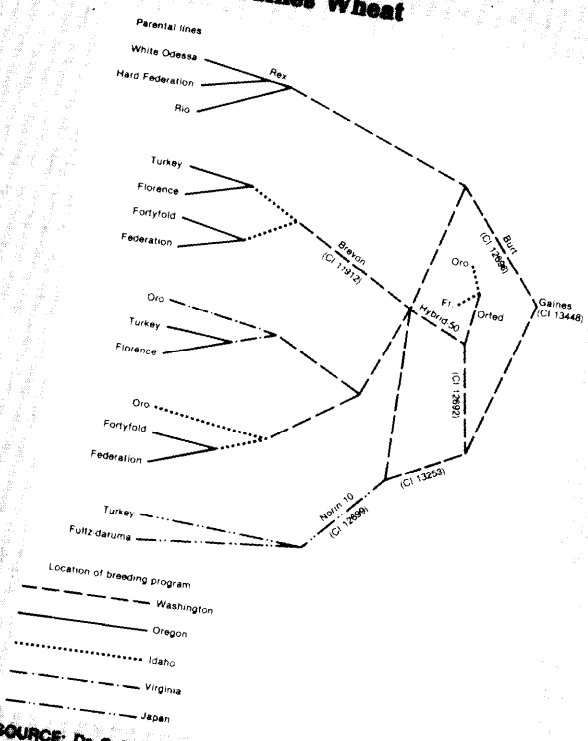
Box 7-C.—Breeding and the Development of Gaines Wheat

The first American soft, white, semi-dwarf, winter wheat variety was released in 1956 and was known by the varietal name "Gaines." It was developed by farmers of the Inland Empire region of the Pacific Northwest—a region of rolling hills and deep soils that included eastern Oregon and Washington and northern Idaho. The flour of Gaines wheat can be used in pastries, cookies, and other soft white wheat products.

Gaines wheat was responsible for major increases in the yields of farmers in the Palouse region in Washington. Fifty years ago, these farms yielded an average of 15 to 17 bushels of wheat per acre. Today, many farms harvest more than 90 bushels per acre. These increases have been the result of a breeding program that dramatically restructured the wheat plant.

High yields of Gaines wheat result from a greater proportion of the plant's energy being channeled into grain production. Moisture and nutrients are more efficiently utilized. Genes that reduce the amount of straw relative to grain produced per plant were incorporated into breeding lines. Short-stature Gaines wheat has not only increased farmers' yields, but it also has served as the main source of genes for the wheat varieties of the Green Revolution for which Dr. Norman Borlaug received the 1970 Nobel Peace Prize.

Wheat is not native to the United States, and thus, the germplasm to develop Gaines had to come from international sources. Many of the necessary breeding stocks were already part of the USDA's National Small Grains Collection, which contains wheats from many countries. Other materials came from individual breeders both in the United States and other countries. The above chart shows the parentage of Gaines wheat and illustrates the numerous crosses and selections that must occur for the development of a crop variety. Today, Gaines has been replaced by improved varieties that were developed from it.



SOURCE: Dr. S. Dietz, USDA-ARS Western Regional Plant, Introduction Station, Pullman, WA.

SOURCE: Adapted from materials provided by Dr. Sam Dietz, Regional Plant Introduction Station, USDA/ARS, Pullman, Washington, 1987.

variation, 2) somatic hybridization, and 3) recombinant DNA technologies.

Somaclonal Variation

During the process of *in vitro* culture of unorganized plant tissues, modifications frequently arise that can be genetically stable and heritable (5,75,90,91). A number of significant somaclonal variants have been isolated from buds produced in unorganized *in vitro* cultures (table 7-5).

However, somaclonal variations may not always persist (74,90). In some cases, variations can be passed on to succeeding generations, but in others they are lost (75,90). In addition to the problem of genetic stability, variant cells

Table 7-5.—Somaclonal Variation in Economically Important Plant Species

Plant	Source of tissue	Characters	Transmission[a]
Oats *(Avena sativa)*	Immature embryo, apical meristem	Plant height, heading date, leaf striping	Seed
Wheat *(Trificurn aesfivurn)*	Immature embryo	Plant height, spike shape, maturity tillering, leaf wax giliadins, amylase	Seed
Rice *(Oryza sativa)*	Seed embryo	Tiller number, panicle size, seed fertility, flowering date, plant height	Seed
Sugarcane *(Saccharum officinale)*	Various	Disease resistance, auricle length, isoenzyme alterations, sugar yield	Vegetable
Corn *(Zea rnays)*	Immature embryo	Endosperm and seedling mutants, pathogen toxin resistance, DNA sequence, changes in mitochondria	Seed
Potato *(Solanurn tuberuosurri)*	Protoplasm leaf callus	Tuber shape, yield, maturity date, plant form, stem, leaf, and flower structure, disease resistance	Vegetable
Tobacco *(Nicotiana tabacum)*	Anthers, protoplasts, leaf callus	Plant height, leaf size, yield grade index, alkaloids, reducing sugars, leaf chlorophyll	Seed
Alfalfa (A4edicago *sativa)*	Immature ovaries	Leaves, petiole length, plant form and height, dry matter yield	Vegetable
Brassicas *(Brassica* spp.)	Anthers, embryos, meristems	Flowering time, growth form, waxiness, glucosinolates, disease tolerance	Seed

aseed = inherited in seeds of variant plants: vegetable = transmitted to clonally reproduced plants

SOURCE W R Scowcroft, S A. Ryan, R I S Brettel, and P J Larkin, "Somaclonal Variation A 'New' Genetic Resource," *Crop Genetic Resources" Conservation and Evaluation,* J H W Holden and J T Williams (eds) (London" George Allen &Unwin, 1984)

and tissues may not regenerate into whole plants or may produce abnormal or sterile plants (75).

Progress in developing somaclonal variation for plant improvement has been promising for a few plant species (5,90,92). However, its general application remains unproven. Further, it is not yet possible to select through *in vitro* culture many valuable traits, such as yield or quality characters. This inability reflects a basic lack of knowledge of the genetic mechanisms controlling many such traits (38).

Somatic Hybridization

A report conducted more than a decade ago on the fusion of leaf protoplasts (cells from which the ceil walls have been enzymatically removed) from two species of tobacco heralded exciting possibilities (11). The process, termed somatic hybridization, held promise of bridging many barriers to hybridization. Questions of whether "impossible hybrids" could be obtained were partially answered with reports of a successful protoplasm fusion from a tomato and potato (72). Unfortunately, as with a hybrid sexually produced 50 years earlier by crossing radish and cabbage, the resulting plant exhibited the least desirable characteristics of each parent and was sterile (75).

Research on irradiation and protoplasm fusion shows promise. By irradiating one set of protoplasts, the genetic material is broken into short sequences, some of which will make its way into the fusion partner. The technique, with considerable development, may eventually enable transfer of genes between sexually incompatible species.

Recent studies show the potential for transferring cellular organelles with their genetic information (chloroplasts and mitochondria) to other species (15,41,75). This technique may be useful in the transfer of genes for the limited number of traits (e.g., photosynthetic efficiency, herbicide tolerance, cytoplasmic male sterility) found in these organelles.

Application of somatic hybridization has been limited to plants from three families: Solonaceae (e.g., potato, tomato, tobacco); Cruciferaceae (e.g., cabbage, rape); and Umbel*liferaceae (e.g.,* carrots) (75). Regeneration of whole plants from protoplasts often remains an obstacle because little is known about the culture conditions needed to cause protoplasts

or undifferentiated tissue to regenerate into whole plants (41,75,111).

Recombinant DNA Technologies

The technologies associated with recombinant DNA allow insertion of specific genetic information into plants to produce altered characteristics. Basic principles of the technologies have been discussed in earlier OTA studies (109,111). Current constraints relate largely to inabilities to culture and regenerate isolated cells of most plant species (41,75).

Genetic engineering techniques may allow scientists to develop, by gene transfer, new agricultural varieties (75), but considerable scientific development is needed before such technologies can become routine. Further, genetic engineering technologies face legal, social, and political questions in light of warnings that potential products might cause health, environmental, or economic problems. With continued research, genetic engineering could augment, but not substantially replace, standard breeding practices.

NEEDS AND OPPORTUNITIES

In the past 10 years, new technologies for germplasm collection, maintenance, and use have been developed (108). Improved germplasm maintenance in the United States will require not only the addition of new technologies, but careful planning for facilities and resources to support them. Determining the appropriateness of a particular technology involves consideration of the biology of the species, the reliability of the technology, the effect of the technology on a collection's composition, and costs. This section discusses several areas of offsite maintenance that need attention and the opportunities for doing so.

Develop a Standard Operating procedure

Studies have only recently begun to systematically address problems of records maintenance, regeneration procedures, seed-drying techniques, storage, liability testing conditions, or improper management (21,30,31,43). This assessment has highlighted numerous appropriate procedures. Implementation of these technologies in the United States and internationally could provide a basis for improving maintenance in offsite collections and developing appropriate avenues for training personnel.

Standard operating procedures for maintaining offsite collections of plants could be developed that include newly developed technologies and incorporate additional procedures as they are developed. Such procedures could be developed by a task force composed of representatives of government, industry, and academia. The task force could specifically consider the use of technologies by the National Plant Germplasm System.

Development of recommendations will not assure improvement of germplasm maintenance in existing U.S. collections. Issues such as the need for additional storage space at NSSL and implementation of better viability testing and regeneration protocols must be addressed by increased funds if necessary. A plan to improve storage and maintenance in NPGS collections should be drawn up, therefore, that would address both the needs for new facilities and support of basic operations. Such a plan could be developed by USDA with or without the suggested task force, or by a separate committee drawn from sectors served by NPGS.

Storage

Cryogenic techniques could greatly extend the storage time of seeds and could reduce costs associated with monitoring seed viability and regenerating samples. USDA funding of research on the effects of cryogenic storage could increase the number of species that can be maintained and allow investigation of concerns about genetic stability.

In *vitro* plants can be used for a range of species with recalcitrant seeds or for those that must be maintained as clones. However, the techniques are not now used extensively for germplasm storage, and uncertainties about genetic stability in the *in* vitro environment have been noted. Cryogenic technologies could be particularly important, but they require further development.

Funds to develop technologies for maintaining plants in offsite collections are already provided through the Agricultural Research Service (ARS) to NPGS researchers. These efforts could be enhanced by making funds available to researchers outside USDA on a competitive basis. As an alternative, the USDA/Competitive Research Grants Office could develop a program that would focus on germplasm maintenance and the application of technology.

Characterization and evaluation of Offsite Collections

Characterization and evaluation data are not available for most plants held by NPGS, but the development of descriptors by crop advisory committees (CACS) (see ch. 9) will provide guidelines for preliminary characterizations of many crops. Technologies for biochemical characterization exist, and consideration should be given to ones that are appropriate for particular crops. Further, careful consideration of the agronomic traits to be evaluated will be necessary.

Improving characterization and evaluation data will require additional funding and personnel. A 10-year NPGS program to provide detailed evaluations of the genetic diversity and potentially useful agronomic characters in cultivated species and their relatives might cost $5 million annually. Such a program would probably require increased collaboration between NPGS facilities and scientists to expand the available expertise, develop a computerized file for each accession, and increase involvement of CACS and breeders in determining which agronomic traits to evaluate.

By examining analyses of the roles of CAC, NPGS facilities, and users of NPGS in recording evaluation data, different ways to improve present efforts might be revealed. Such an examination could be performed by an expert committee appointed by USDA. Recommendations could include specific roles for components of NPGS and mechanisms for accomplishing those goals.

Grant funds could be made available through ARS to researchers and breeders screening for particular traits. Such funds could encourage greater use of germplasm collections as well as increase the information about accessions. Data from evaluations could then become part of the permanent GRIN record.

Maitenance of Endangered wild Species

The efforts of botanic gardens and arboretums to obtain and store seeds or plants of endangered wild species have only recently been coordinated. Additional funding for facilities and personnel to develop and maintain such collections will be needed. Further, each species presents a potentially unique set of requirements for maintenance and regeneration that must be taken into account.

Funds have come in part from the Institute for Museum Services (34). They have been used for daily operations as well as to establish storage facilities. Continued funding could provide for the maintenance of many endangered wild plants, However, it has been estimated that maintaining the 3,000 or so rare and endangered plant taxa will cost at least $1.2 million annually *(71).*

One possibility is to expand the scope of NPGS activities to include endangered wild species. NPGS personnel have considerable expertise in offsite maintenance of plants, and including endangered wild plants as a responsibility would take advantage of this expertise. However, an enlargement of NPGS'S scope would require additional funding for personnel and facilities. And because responsibilities are currently divided among various parts of NPGS on a crop-by-crop basis, an administrative mechanism for assigning responsibility for a particular species would be needed.

As an alternative, an existing private organization, such as the Center for Plant Conservation (CPC), could become the mechanism within NPGS for coordinating maintenance of endangered wild plants. Funds could be designated through USDA/ARS for this purpose, and CPC could be responsible for coordinating efforts and administering funds to cooperating botanic gardens and arboretums.

Improve Movement of Germplasm Through Quarantine

Technologies that identify viruses in imported plants could reduce delays associated with the testing of a few plant species. Although many potentially useful technologies exist, few are applied routinely to quarantine testing, because USDA's Animal and Plant Health Inspection Service (APHIS) lacks sufficient trained personnel, facilities, or operating funds needed to implement a particular technology. Cooperation between APHIS and NPGS facilities could enhance the technical expertise applied to quarantine-testing and other solutions to improve quarantine efforts.

A panel representing APHIS, the research community, and NPGS could be convened to assess the adequacy of facilities and programs relating to quarantine. It could make recommendations for implementing newer technologies, improving present facilities, constructing new facilities, and mechanisms for promoting cooperation with NPGS facilities. The panel could also redirect existing budgets within USDA to address specific problems and, if necessary, develop legislation for increasing USDA appropriations to meet quarantine needs. The panel might also consider mechanisms for incorporating new technologies and the appropriateness of facilities and personnel for performing them.

Promote Basic Research on Maintenance and Use of Plant Germplasm

Although technologies to maintain plants offsite have advanced considerably in recent years, several fundamental questions still need to be addressed.

In the past, storage has essentially referred to orthodox seed storage. It is increasingly apparent that new techniques for storage of nontraditional forms of germplasm (e. g., recalcitrant seeds, pollen, and in vitro cultures) are needed. Although cryogenic storage has been used for several years on animals, its use with plants has only recently been investigated. Questions about the nature of genetic control and the mechanisms involved in somaclonal variation are as yet unresolved. These new storage technologies all require better understanding of developmental processes, of cell and seed physiology, and of mechanisms of cellular deterioration and repair.

New methods for storage of naked DNA and RNA and possible recovery of DNA from dead cells could lead to a new concept in germplasm conservation. Caution must be exercised, however, to ensure that limited funds are not disproportionately channeled into this high-technology area. If genetic conservation is a goal, then existing technologies and those showing promise should receive adequate funding before more speculative approaches are pursued.

Improved understanding of the biochemical, genetic, and physiological control of development may lead to techniques for characterizing and evaluating germplasm. The genetic control of most important traits is not yet understood. Additional research on the basic structure and function of genes can also improve the biological knowledge necessary for genetic manipulation of plants.

Funding for research on germplasm has come from several agencies. But research priorities at the National Science Foundation (NSF) or USDA's Competitive Research Grants Office (CRGO), however, generally do not encompass projects that focus on germplasm maintenance. Perhaps a new program within USDA/CRGO or NSF could be created to address research appropriate to germplasm maintenance and use.

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