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ABSTRACT

Landraces and wild relatives of crops from centers of diversity have been rich sources of resistance to new pathogens, insect pests, and other stresses as well as for traits to improve food and fiber quality, animal feed, and industrial products. Because very few crops grown in the U.S. are native, plant introductions are vital to our agriculture. The National Plant Germplasm System (NPGS) was established to acquire, preserve, and distribute plant genetic resources from around the world so that scientists have immediate access to these source materials. The active collection is maintained and distributed by 19 national germplasm repositories. The base collection is preserved at -18°C at the National Seed Storage Laboratory. The NPGS's genetic resources are made freely available to all *bona fide* users for the benefit of humankind. Recent international agreements such as the Biodiversity Convention will impact acquisition and exchange of germplasm, but the NPGS goal is to maintain the germplasm exchange critical to feeding the increasing world population in the future.

INTRODUCTION

Landraces and wild relatives of crops from centers of diversity have been rich sources of resistance to new pathogens, insect pests, and other stresses as well as for traits to improve food and fiber quality, animal feed, and industrial products. This valuable genetic diversity has resulted from evolutionary processes including mutation, recombination, natural selection, migration, and genetic drift in many ecological niches. Human intervention has produced both positive and negative effects on diversity.

No country has all of the plant genetic resources required to develop and maintain a high level of agricultural productivity. The U.S. has an extremely limited number of native species of economic importance including some grasses, sunflower, cranberry, blueberry, strawberry, pecan, and a few other species. As with many countries, our exceptionally productive agricultural systems were founded on introduced plant genetic resources.

Immigrants from Europe and Asia brought seed with them. Prior to that, native North Americans had introduced maize, beans, squash and other crops from Central and South America. In 1819 American consuls overseas were asked to collect seeds of useful plants. The U.S. Patent Commissioner administered the introduction of plants from 1836 to 1862. The continuing need to acquire and introduce plant germplasm into the U.S. was one of the reasons for establishing the U.S. Department of Agriculture (USDA). The Organic Act, of 1862, establishing the Department of Agriculture, directed the first Commissioner of Agriculture, Isaac Newton, "to collect, as he may be able, new and valuable seeds and plants; to test, by cultivation the value of such of them as may require such tests; to propagate such as may be worthy of propagation, and to distribute them among agriculturists." In 1898, the Seed and Plant Introduction Section, which later became the Plant Introduction Office, was established to manage plant explorations and introductions.

Before the late 1940's, introductions were sent directly to interested scientists without any requirement that they be maintained. Adequate preservation methodologies and facilities were not available, and many accessions were lost.

Landraces and wild relatives are useful sources of genetic diversity to meet plant breeders' needs. But, as farmers in centers of diversity switch to new stress tolerant, higher yielding cultivars, these valuable sources of useful genes will be lost forever unless they have been collected and preserved *ex situ* in gene banks.

EX SITU CONSERVATION STRATEGIES

Ex situ collections of germplasm can be maintained as 1) living and growing organisms and 2) living but quiescent organisms (Eberhart et al., 1995). Examples of living and growing collections include field and screen house collections, botanical gardens, and cell and tissue cultures.

In the living but quiescent collections, organisms are stored in a state of "suspended animation." Examples are seeds and cryopreserved tissues and cultures in gene banks. Only orthodox seeds (those which are tolerant to desiccation) and dormant vegetative buds from apple are currently stored in quiescent collections, but the technology is developing rapidly that will permit the preservation of most forms of plant germplasm.

Technological demands are minimal in living and growing collections. However, these collections are expensive in terms of labor and space. Most importantly, living and growing collections may be susceptible to frosts, droughts, diseases, insects, and other disasters. Collections of living but quiescent organisms and tissues provide a low risk backup to the living and growing collections. Once in storage, preserved organisms require minimal space and labor, and this permits the preservation of many collections.

Technologies for preserving *Ex Situ* Collections

The basic principle of preserving quiescent biological tissues is to limit biochemical changes that are caused by either metabolism or the stochastic processes of aging. For many biological materials, the procedures used to limit chemical reactions (dehydrating and/or freezing) are lethal. Thus, these preservation procedures cannot be used in certain quiescent *ex situ* collections. There are many different types of tissues than can be used as propagules. Seeds and pollen are propagules that are sexually derived from plants, whereas propagules such as vegetative buds, shoot tips, somatic embryos, cell suspensions, and root tissues are asexually derived. For purposes of conserving genetic diversity, the choice of propagule depends on the ease in which it can be preserved and whether particular gene combinations are desired.

For seeds, we distinguish between 'orthodox' and 'recalcitrant' types. Orthodox seeds are easily stored, while recalcitrant seeds are more difficult to store. Fortunately, many crops important to U.S. agriculture form orthodox seeds. Triticeae cereal and grass seeds have good longevity in storage (Harrington, 1972; Priestley et al., 1985), and some have been reported to survive more than 100 years (Roos, 1986). However, a number of crop species (e.g., wild rice, citrus, avocado, mango, cacao, coffee) and several tree species (e.g., oak, maple, buckeye) produce recalcitrant seeds. The basic distinction between orthodox and recalcitrant seeds lies in their relative ability to survive desiccation.

Preservation of Orthodox Seeds

The technologies for preserving orthodox seeds are well understood for the most part. Seeds should be dried and stored at a low temperature (Justice and Bass, 1978). Research by Justice and Bass (1978), Bass (1980), and Bass and Stanwood (1978) showed that reducing the storage temperature from 5°C to sub-zero temperatures increased seed longevity from less than 10 years for some species to several decades for most species.

The ultra-low temperature of liquid nitrogen used in cryogenic storage should extend seed longevity (Stanwood, 1980, 1985). After 10 years of cryogenic storage, no major differences in viability were observed between onion seeds stored at -18°C and liquid nitrogen temperatures (Stanwood and Sowa, 1995). However, major differences were observed between the sub-zero temperatures and 5°C. The protocols for cryogenic storage of orthodox seeds were established by Stanwood and Bass (1981). Seeds are stored in the vapor phase above liquid nitrogen (approximately -160°C). The choice between using conventional storage at -18°C or storage at liquid nitrogen temperatures depends on whether 1) the accession shows damage during initial exposure to liquid nitrogen, 2) the species produces large seeds (annual operating cost per sample in liquid nitrogen is at least three times higher than conventional storage at -18°C), and 3) the longevity characteristics of the species.

Although seed drying extends longevity, there are limits to the beneficial effects; and the optimum moisture content varies with the chemical composition of the seed (Vertucci and Roos, 1990, 1993; Vertucci et al., 1994; Ellis et al., 1989, 1990). Drying seeds beyond a critical moisture content can result in accelerated deterioration at above zero temperatures. Using basic thermodynamic principles, scientists at the NSSL (Vertucci and Roos, 1990, 1993; Vertucci et al., 1994) have established that, contrary to the viability equations (Ellis and Roberts, 1980; Ellis et al., 1989), the effects of storage temperature and water content of seeds are not independent. Consequently, the optimum water content for seed storage varies both with the seed species and with the temperature of storage. Clearly, there are insufficient funds to determine the specific optimum moisture content for each of the 8,000 species represented in the NPGS collection. However, the thermodynamic principles used by Vertucci and Roos (1990, 1993) and Vertucci et al. (1994) can be used to predict optimum moisture levels for all orthodox seeds at all storage temperatures. Based on the finding that 25% RH provides the optimum moisture level for storage at 25°C for all orthodox seeds studied, the optimum water content at any other storage temperature can be calculated. This procedure has eliminated the requirement of determining moisture contents for each accession and saves approximately two hours of processing time for each seed sample.

Preservation of Orthodox Pollen

Pollen from many plant species can be preserved using the same principles that are used for orthodox seeds (Connor and Towill, 1993). Preservation of pollen produced from long-lived perennial plants is especially

useful for the plant breeder. Pollen storage requires little space and labor. Like orthodox seeds, preservation of pollen in living but quiescent collections can serve as a backup for living and growing *ex situ* collections (Towill, 1985; Connor and Towill, 1993).

Preservation of Desiccation-Sensitive Propagules

Unlike most biological tissues, orthodox embryos and pollen tolerate severe dehydration; this ability makes them amenable to storage in quiescent collections. Desiccation-sensitive tissues cannot be easily stored at sub-freezing temperatures because the water that is necessary for their survival freezes with lethal consequences. A number of methods by which tissues can be exposed to sub-freezing temperatures without lethal ice formation have been developed. These methods involve optimizing the water content and then cooling tissues to the desired temperature at an appropriate rate. Two methods of handling recalcitrant seed have given results varying from excellent (80%) (Vertucci et al., 1991; Wesley-Smith et al., 1992; Vertucci et al., 1993) to mediocre (30-50%) (Wesley-Smith et al., 1993). Survival rates depend largely on the species and its developmental status. The first method is applicable to those tissues that can survive water contents as low as 0.3 g H_2O/g dw (30% seed moisture) or water potentials as low as -15 MPa. In this method, the moisture content and temperature are optimized so that both desiccation and freezing damages are avoided (Vertucci, 1989; Vertucci et al., 1991, 1995). The critical moisture content for desiccation damage increases as temperature is reduced (Vertucci et al., 1995). Thus, the window of survivable moisture levels narrows as the storage temperature declines (Vertucci et al., 1991, 1993, 1995). This method is presently being adopted for long term storage of recalcitrant seeds of wild rice (*Zizania palustris*)(Vertucci et al., 1995).

The second method for preserving recalcitrant seeds is used for embryos which are extremely sensitive to dehydration and cannot survive water contents lower than about 0.6 g H_2O/g dw (60% seed moisture). These materials must be preserved in the "vitrified" state (Wesley-Smith et al., 1992).

Similar cryopreservation procedures have been used with other propagules with variable success rates. Survival rates of 0 to 80% after exposure to liquid nitrogen can be obtained for vegetative buds of apple (Towill, 1990; Forsline et al., 1993) and apical shoot tips of sweet potato (Towill and Jarrett, 1992), survival being largely dependent on genetic constitution and developmental stage.

In vitrified samples with high moisture contents, lethal ice crystals do not form even though samples are stored at sub-freezing temperatures (Fahy et al., 1984). Ice is prevented because the samples are treated with cryoprotectants and then cooled at such a fast rate that ice crystals do not have time to form. The solution in these samples becomes a glass. There are several steps required for successful cryopreservation through vitrification (Towill, 1990). First, a stable glass must be created. This is usually accomplished by loading cells with protectants, and then adjusting the water content of cells to optimal levels which limit desiccation damage but encourage glass formation. The sample must then be cooled appropriately, and this usually means at extremely fast rates.

The protectants that are used in cryopreservation have two purposes: 1) to prevent cell constituents from denaturation during the desiccation phase and 2) to stabilize the glass. Research shows that many plant systems naturally accumulate these protectants during particular developmental stages. For example, during fall, winter-hardy woody tissues acclimate and become more tolerant to sub-freezing temperatures. Also, during maturation, orthodox seeds accumulate massive quantities of sugars and proteins believed to be protectants against various stresses. Scientists at the NSSL and elsewhere are studying the mechanisms of protection with the objective of incorporating these chemicals into tissues that do not accumulate them naturally (Towill and Jarrett, 1992). Non-natural protectants such as dimethyl-sulfoxide (DMSO) and ethylene glycol are also commonly used.

Vitrified samples must be stored at temperatures where the glass cannot "melt." This necessitates storage at very low temperatures, either directly in liquid nitrogen (-196°C) or in the vapor above liquid nitrogen (about -160°C)(Fahy et al., 1984). Thawing cryopreserved samples is critical also and is usually done rapidly to avoid formation of ice. When samples are retrieved from storage, they are grown in culture and then transplanted or grafted onto existing stocks. The success of a certain cryopreservation treatment is evaluated by the proportion of propagules that develop into growing plants.

THE NATIONAL PLANT GERMPLASM SYSTEM

The Research and Marketing Act of 1946 (Public Law 733) authorized the creation of four Regional Plant Introduction Stations (Ames, Iowa; Pullman, Washington; Geneva, New York; Griffin, Georgia) with the mission to acquire, maintain, evaluate, and distribute germplasm to scientists to be used for crop improvement. The National Small Grains Collection, now in Aberdeen, Idaho, began in 1894 as a breeder's collection in Beltsville, Maryland. The Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin, was established in 1947. National Clonal Germplasm Repositories were established in the mid-1980s to provide more systematic maintenance of vegetatively propagated germplasm. These repositories grow and maintain the active collections and distribute

samples to scientists worldwide. The National Seed Storage Laboratory (NSSL), Fort Collins, Colorado was dedicated in 1958 as a long-term storage facility to preserve the base collection for backup of the active collections.

These units have been integrated into a National Plant Germplasm System (NPGS) (ARS Information Service, 1990; Shands et al., 1989). The NPGS is a network of cooperating institutions, agencies, and research units in the Federal, State, and private sectors. The mission of the NPGS is: "To effectively collect, document, preserve, evaluate, enhance, and distribute plant genetic resources for continued improvement in the quality and production of economic crops important to U.S. and world agriculture. This is achieved through a coordinated effort by the U.S. Department of Agriculture in cooperation with other public and private U.S. and international organizations. Plant genetic resources in the NPGS are made freely available to all *bona fide* users for the benefit of humankind."

In addition to the active and base collections in NPGS, plant breeders maintain working collections of plant materials used in their programs. As cultivars, parental lines, and elite germplasm are developed, released, and registered, these are entered in the NPGS active and base collections.

In the National Plant Germplasm System, the four Regional Plant Introduction Stations, the National Clonal Germplasm Repositories, the Inter-regional Potato Introduction Station, the National Small Grain Collection, specific crop collections, and the Woody Landscape Collection of the National Arboretum each functions, and is accepted, as a national plant germplasm repository even though some are partially supported by regional and inter-regional funds. The more than 440,000 accessions maintained in the NPGS active collections have been divided among these 19 repositories.

These repositories cooperate and participate in a coordinated national program of acquiring and exchanging foreign and domestic plant germplasm potentially valuable for agricultural, horticultural, medicinal, industrial and environmental uses. The new acquisitions must be increased, characterized, and preserved as part of the active collection. Each repository conducts a systematic evaluation program to obtain specific information on disease and insect resistance, nutritional quality, agronomic and physiological attributes, and other traits of interest. Information on the collection and characterization (passport data) and evaluation data are entered in the Germplasm Resources Information Network (GRIN). When requested, samples are distributed to scientists worldwide at no cost for use in crop improvement and basic research. Research relating to improved methods of collection, regeneration, propagation, preservation, evaluation, and distribution is conducted, and the results are published.

The National Germplasm Resources Laboratory (NGRL) located at the Beltsville Agricultural Research Center (BARC), Beltsville, MD, is responsible for a number of activities that support the entire NPGS.

The Plant Introduction Office (PIO) coordinates the acquisition and exchange of plant germplasm; documents passport data and descriptive information for newly acquired material and assigns unique Plant Introduction (PI) numbers; publishes an annual USDA Plant Inventory of newly received accessions; and serves as a liaison on quarantine matters. Plant germplasm for the NPGS is acquired through exchanges, exploration (domestic and foreign), special projects and agreements, gifts, and travelers. In addition to introduced germplasm, all released plant materials (cultivars, germplasm releases, parental lines, and genetic stocks) that are registered by the Crop Science Society of America are assigned PI numbers and the seed is deposited in the appropriate active collection and the NSSL by the originator.

The Plant Exploration Office (PEO) works with germplasm curators, Crop Advisory Committees (CAC), state universities and others to assess the genetic diversity in germplasm collections currently held by the NPGS and others as compared to total genetic diversity that may exist in nature. This assessment is used to develop long-range strategies for increasing the genetic diversity of U.S. collections. Based on these strategies, gaps in current germplasm collections are identified and communicated to the appropriate CAC or to other crop specialists for their concurrence. Priorities for exploration are influenced by several factors such as the completeness of the U.S. collection, the need for specific traits of agricultural significance, the threat of immediate loss of old landraces and wild relatives in centers of diversity because of agricultural changes or urban development, and political factors affecting future availability of germplasm.

The Germplasm Resources Information Network (GRIN) is the official database of the NPGS and is maintained on a computer in the National Agricultural Library at Beltsville, Maryland. The functions of the GRIN database for the NPGS are to: 1) act as a repository of all information on NPGS plant germplasm, 2) unify the NPGS with regard to data standards and coordinate the movement of germplasm, 3) allow users of the germplasm fast access to the most current data available, 4) facilitate and track the distribution of germplasm, and 5) provide to germplasm maintenance sites a system of inventory management that automatically signals the need for germplasm regeneration.

Data in GRIN are available to any plant scientist or researcher worldwide, either through direct connection to the database or through contact with the curator for the active collection of the crop of interest. GRIN contains data on taxonomy, origin, evaluation and characterization for plant germplasm preserved in the NPGS. All

movements and distributions of germplasm within the NPGS and foreign countries are recorded in GRIN.

All plant germplasm entering the NPGS from outside the U.S. must comply with federal quarantine regulations, which are designed to facilitate the exchange of plant germplasm while limiting/ preventing the movement of pathogens. Regulations are written, interpreted, and enforced by APHIS. Scientists cooperate to import plant germplasm free of pests. Accessions of certain crops must be grown under quarantine at designated sites under APHIS inspection, including greenhouses at specified locations and the ARS St. Croix research station, before they can enter the NPGS active and base collections.

The NGRL facilitates the activities of Crop Advisory Committees. The public and private scientists on these committees represent the germplasm user community for a particular crop or a group of crops. These committees provide crop-specific expert guidance on germplasm needs, collection gaps, descriptors, documentation, regeneration, evaluation, and research goals to various components of the NPGS.

Although the ARS components of the NPGS are administered by the Area Director for the geographic location of that component, the Associate Deputy Administrator for Genetic Resources and the National Program Leader for Plant Germplasm on the National Program Staff provide leadership for the NPGS and coordinate activities. They also provide administrative support to the various advisory boards and committees for plant genetic resources.

Plant germplasm collections in NPGS include older and current crop cultivars, elite breeding lines, landraces of crops that have emerged over millennia through selection by farmers, wild and weedy plants related to cultivated crops, and genetic stocks maintained for research.

The active collections of *Hordeum, Secale, Triticum, Aegilops, X Triticosecale, Avena*, and *Oryza* are maintained and distributed by staff of the National Small Grains Collection (NSGC), Aberdeen, Idaho. More than 132,000 samples of seed were distributed during 1993, including about 14,000 to U.S. scientists, 10,000 to foreign scientists, and 78,000 to cooperators for germplasm evaluation. More than 1.4 million evaluation records representing 152 descriptors are available on GRIN for the small grains collections. Regenerations are grown in field and greenhouse nurseries at Aberdeen, Idaho; Maricopa, Arizona; and Stuttgart, Arkansas (rice). The 3,011 new accessions added in 1993 included barley from China and Nepal, various small grains from Russia and Georgia, *Aegilops* from Turkey, Israel, and Syria, and wheat from Turkey.

The active collections of Triticeae species other than small grains are maintained and distributed by staff at the Regional Plant Introduction Station, Pullman, Washington. During 1993, 448 new accessions were added; and 1154 samples of various Triticeae grasses were distributed. Regenerations are completed at the Pullman and Central Ferry farms. The NPGS holdings of Triticeae species are shown in Table 1.

As accessions propagated by seeds are regenerated or increased at the repositories, seed samples are divided with part staying in the local active collection and the other part deposited in the NSSL base collection. The principal mission of NSSL is to preserve the base collection of the NPGS and to conduct research to develop new and improved technologies for the preservation of seed and other plant propagules. The goal of NSSL is long-term preservation of back-up samples of all accessions maintained in active collections at national plant germplasm repositories. The NSSL facility was expanded fourfold and modernized in 1992. The new storage vaults have the capacity to store and protect more than one million samples.

Seed samples received at NSSL are dried, counted, tested for viability and placed in moisture-resistant containers in sub-zero cold vaults (-18°C) or stored above liquid nitrogen (-160°C) in cryotanks. Samples are monitored periodically for viability, and sub-standard samples are regenerated by the appropriate repository.

Minimizing genetic change during *ex situ* preservation is paramount to retain as much genetic variation as possible for future use (Crossa et al., 1994). For seed, a key first step to minimize genetic change is to preserve the initial regenerated sample in the base collection. This regeneration should be done with an appropriate number of plants with the required pollen control under optimum growing conditions to produce high quality seed. Careful processing and drying are required to maintain high viability. Storage of dry, high quality seed at sub-zero temperatures can extend viability for many years before a second regeneration of the base collection is necessary. When continuing demand on the active collection occurs, seed from the base collection should be used for every second or third regeneration.

Plant germplasm preservation research at NSSL focuses on the development of new and improved technologies for the long-term preservation of all forms of plant germplasm. This research is expected to increase: 1) the number of species that can be stored at NSSL, 2) the longevity of the various accessions, and 3) the efficiency of viability testing of accessions. Longer storage periods and reduced number of field and/or greenhouse regeneration cycles will result in lower costs and greater genetic integrity of the germplasm. Research of the Plant Germplasm Preservation Research Unit at the NSSL is addressing also questions on the nature of seed aging under dry conditions and low temperatures, how the rate of deterioration can be predicted and monitored efficiently, and how the effects of aging can be reversed. Research scientists at NSSL work closely with all components of NPGS.

Table 1. National Plant Germplasm System Holding of Triticeae*

Species	Common Name	Numbers of Accessions
Aegilops spp.		3672
Agropyron cristatum	Fairway c crested wheatgrass	298
Agropyron desertorum	Standard crested wheatgrass	107
Agropyron fragile	Siberian crested wheatgrass	83
Agropyron spp.		167
Amblyopyrum muticum		12
Elymus canadensis	Canada wildrye	45
Elymus caninus	Bearded couch	43
Elymus dahuricus	Dahurian wildrye	168
Elymus elymoides	Squirreltail	10
Elymus glaucus	Blue wildrye	44
Elymus lanceolatus subsp. *lanceolatus*	Thickspike wheatgrass	32
Elymus lanceolatus subsp. *wawawaiensis*	Snake river wheatgrass	40
Elymus sibiricus		147
Elymus trachycaulus subsp. subsecundus	Beaded wheatgrass	33
Elymus trachycaulus subsp. *trachycaulus*	Slender wheatgrass	4
Elymus trachycaulus subsp. *violaceus*	Violet wheatgrass	6
Elymus villosus	Hairy wildrye	2
Elymus viginicus	Virginia wildrye	40
Elymus spp.		1102
Elytrigia repens	Couchgrass. Quackgrass	149
Elytrigia spp.		464
Eremopyrum spp.		35
Heteranthelium spp.		38
Hordelymus europaeus		7
Hordeum vulgare subsp. *vulgare*	Barley	26013
Hordeum spp.		2084
Leymus angustus	Altai wildrye	218
Leymus arenarius	Beach wildrye	24
Leymus cinereus	Great Bain wildrye	78
Leymus condensatus	Giant wildrye	2
Leymus millis	Beach wildrye	7
Leymus racemosus	Mamoth wildrye	24
Leymus spp.		145
Pascopyrum smithii	Western wheatgrass	50
Psathyrostachys juncea	Russian wildrye	165
Psathyrostachys spp.		15
Pseudoroegneria spicata	Nluebunch wheatgrass	137
Pseudoroegneria sppl		101
Secale cereale subsp. *cerale*	Rye	1806
Secale spp.		125
Taeniatherum spp.	Medusa's-head rye	27
Thinopyrum ponticum (Elytrigia elongata)	Tall wheatgrass	74
Thinopyrum intermedia (Elytrigia inter.)	Intermediate wheatgrass	156
Triticum aestivum	Common Wheat	34603
Triticum dicoccoides		1670
Triticum dicocconemmer		533
Triticum durum	durum wheat	6832
Triticum monococcum	einkorn wheat	235
Triticum spelta	spelts	1179
Triticum timopheevii		51
Triticum timopheevii var. *araraticum*		268
Triticum turgidum		460
Triticum spp.		2058
Total		85907

*spp. is a grouping of species not listed separately, or accessions not yet classifed.

The NPGS maintains one of the largest *ex situ* collections of plant genetic resources in the world. A detailed report of the NPGS history, policies, and architecture is given in *Plant Breeding Reviews* (ed. by J. Janick, 1989). Since 1898, about 575,000 accessions with real or potential economic importance to U.S. agriculture have been acquired through the Plant Introduction Office. Many of these are among the more than 440,000 accessions, representing over 8,000 plant species, that are now preserved in the NPGS.

The NPGS has been described as a "user-driven system." Between 1986 and 1992, the NPGS distributed an average of 175,400 samples each year to U.S. public scientists (67%), U.S. private industry scientists (12%), foreign public scientists (9%), foreign private industry scientists (10%), and international centers and USAID (2%).

CORE SUBSETS

When a scientist determines that there is inadequate genetic variation in available germplasm for a desired attribute, new accessions are needed that will provide the highest probability of identifying useful source materials with minimum screening. Sometimes this can be achieved by obtaining accessions from an area where the problem has been endemic for many years; e.g., low soil pH. A list of candidate accessions can often be generated when appropriate information is in the database.

In other cases, especially for new pathogen strains or insect biotypes, searching database information is of little or no value. When the scientist must search within the crop collection for the desired trait, an initial screening of a smaller, diverse subset may reduce time and costs. The idea of developing such a subset was proposed by Frankel (1984) and further developed by Brown (1989a,b, 1995). They suggest that "A core collection consists of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum of the whole collection. The core should include as much as possible of its genetic diversity. The remaining accessions in the collection are called the reserve collection." The core subset is suggested to be about 10% of the crop collection, but may vary from 5% for very large collections to 50% or more for very small collections, with about 3,000 suggested as a maximum number.

Brown (1989a) recommended stratified sampling methods when establishing core collections. Grouping begins with taxonomic affinity (e.g., species, subspecies, cytological races). Accessions within each taxon can be then assigned to strata based on ecogeographic zones and genetic characteristics (e.g., ploidy level, photoperiod response, races, etc.). Groups such as races of maize (based primarily on ear morphology) may be preferable to country of origin for defining groups because geopolitical boundaries often are incongruent with ecogeographic niches. In other crops, country of origin (or region of adjacent countries) may be the only available means for developing preliminary groups.

Development of a useful core subset may involve the following steps: 1) assembling and reviewing passport data and other information to be used in establishing non-overlapping groups, 2) assigning accessions to appropriate groups, 3) choosing accessions for the preliminary core subset from each group, and 4) collecting data on phenotypic and genetic traits for accessions in the preliminary core and using multivariate analytical methods to construct clusters and dendrograms to elucidate systematic and statistical genetic relations for further refinement of the core subset.

When funding is available to characterize and statistically analyze the entire crop collection for several descriptors, steps 2, 3, and 4 can be conducted simultaneously. Assigning heavier weights to genetic markers and highly heritable phenotypic traits may improve clustering. Groups generated as clusters from statistical analyses of the data will usually be the most robust. If only a few descriptors were analyzed initially, additional descriptors may be measured for the preliminary core, and then step 4 repeated with data from all available descriptors. When financial resources are limiting or very large numbers of accessions must be characterized, steps 2, 3, and 4 will need to be completed sequentially.

Proportional sampling within each group may provide a more representative sample of the total genetic diversity in the core subset than would a completely random sampling from the crop collection. Once the number needed from each group has been determined, accessions for the core subset are usually chosen randomly within each group. However, some curators are choosing accessions with more desirable agronomic traits within each group.

Clusters generated by multivariate analyses may provide a better understanding of patterns of genetic divergence and diversity and will often identify ecogeographic regions that have not been adequately sampled, especially when the origin of each accession in the core is plotted geographically. This information may be valuable in planning future acquisitions.

The core collection concept has gained wide acceptance and core collections are being developed in many countries (Hodgkin et al., 1995; Knüpffer and van Hintum, 1995). The NPGS is developing a core subset for each of the major crop collections (Erskine and Muehlbauer, 1991; Holbrook et al., 1993; Diwan et al., 1994). The core subset will then be used for more extensive characterization and evaluation. The reserve subset will be maintained as an important part of the NPGS base and active collections.

The core subset is expected to remain dynamic with addition, deletion, and substitution of accessions as additional pertinent information becomes available and as new accessions are acquired. Nevertheless, with time, changes to the core should decrease in frequency and magnitude.

INTELLECTUAL PROPERTY RIGHTS

The U.S. Plant Variety Protection Act (PVPA) requires that a sample of each protected cultivar be stored at the National Seed Storage Laboratory. These voucher samples are not to be distributed. However, the owner has the option to provide a second sample to the NPGS base and active collections of NPGS which provides easy access by users for research purposes. Patented plant materials are not accepted by the NPGS. Accessions in the NPGS base and active collections are not eligible for PVP or for patents.

INTERNATIONAL COOPERATION AND COORDINATION

The need to preserve, exchange, and utilize plant genetic resources is recognized worldwide. Even countries with great genetic diversity in certain crops are heavily dependent on many crops introduced from other areas. Because the U.S. has had to import nearly all of its crop germplasm, the NPGS maintains a very comprehensive germplasm collection from around the world. The NPGS has assisted several countries in recovering germplasm of their key crops, which had been lost for various reasons. Many countries now have genetic resource preservation programs with an associated gene bank. IPGRI indicates that the number of gene banks worldwide holding *ex situ* collections is 1060 (personal communication). Several of these, in addition to NSSL, were part of the former IBPGR network of designated base collections (Table 2). The NPGS maintains a close working relation with many of these programs and freely exchanges germplasm.

The International Maize and Wheat Improvement Center (CIMMYT) is developing the International Wheat Information System that will integrate and make available data from three major components: the Wheat Management System; the Wheat Germplasm Bank System; and the Wheat Data Management System. The software will have general application to self-pollinated crops. This system may serve as a model and become part of a CGIAR systemwide database improvement project coordinated by IPGRI that will facilitate the consolidation and exchange of information and genetic resources.

The development and adoption of the Convention on Biological Diversity, however, will alter procedures and policies for germplasm exchange. The objectives of the Convention (as stated in Article 1) are "the conservation of biological diversity, the sustainable utilization of its components, and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources by appropriate access to genetic resources, and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to those technologies, and by appropriate funding". Article 15, Access to Genetic Resources, reaffirms the sovereign rights of States over their natural resources. It also states that States shall endeavor to create conditions to facilitate access to genetic resources and not to impose restrictions which run counter to the objectives of this Convention. However, access shall be on mutually agreed terms. The Convention provides for sharing benefits derived from shared genetic resources with the country of origin, or the

Table 2. Genebanks with Base Collections of Triticeae Species*

Institution	Species	Number of Accessions
CAAS (China)	*Triticum*	*10,427*
CIMMYT (Mexico)	*Triticum*	73,794
CNR (Italy)	*Triticum*	32,000
ICARDA (Syria)	*Triticum, Hordeum, Triticale*	50,255
NGB (Sweden)	*Hordeum, Secale*	14,410
NIAR (Japan)	*Hordeum*	6,236
PGI (Japan)	*Aegilops*	6,774
PGR (Canada)	*Hordeum*	2,200
BG/PAS (Poland)	*Secale*	1,320
PGRC/E (Ethiopia)	*Hordeum*	12,648
VIR (Russia)	*Triticum*	73,082

*Most of these were part of the former IBPGR network of designated base collections (Number of accessions were provided by IPGRI).

country providing such resources where they have been acquired in accordance with the Convention. *Ex situ* collections located outside of the country of origin that were acquired prior to the entry into force of the Convention (December, 29, 1993) do not fall under the terms of the Convention.

In order to comply with the Convention, it is expected that material transfer agreements (MTAs) will be required to accept and to distribute accessions acquired after December 29, 1993. The form of these MTAs for the NPGS is still under development. Possible requirements of a recipient might be as follows: 1) to report to both the donor and NPGS any evaluation results, 2) to acknowledge the germplasm contribution (indicating source country) in publications and variety releases, and 3) to negotiate a license with the donor in the event derived products are developed that have potential commercial value. Although NPGS will need to notify the source nation when an accession subject to a MTA is distributed, the responsibility for monitoring commercial developments by the recipient is expected to remain with the source country, since NPGS has no charter or funding to be a collector or agent.

SUMMARY

Because very few crops grown in the U.S. are native, plant introductions have been vital to our agriculture. The development of a comprehensive NPGS for *ex situ* preservation of plant genetic resources obtained from around the world was necessary to provide scientists with source materials for their programs.

Technologies required to preserve genetic resource propagules in *ex situ* collections in a living but quiescent form are being developed and refined. In the past decade, there have been major technological advances which permits living organisms to be preserved in "suspended animation." This technology will enable us to store our valuable genetic resources safely and efficiently.The more than 440,000 accessions maintained by the NPGS include local landrace collections, improved cultivars, wild crop relatives, and genetic stocks. The active collection is maintained and distributed by nineteen national plant germplasm repositories. The base collections for seed crops are preserved at sub-zero temperatures at the National Seed Storage Laboratory, Fort Collins, Colorado. Plant genetic resources of the NPGS are made freely available to all *bona fide* users for the benefit of humankind. Between 1986 and 1992, an average of 175,400 samples per year were distributed worldwide by NPGS.

It is important that genetic changes during *ex situ* preservation are minimized. The procedures now used by the NPGS for orthodox seeds include regenerating a high quality initial sample for the base collection, carefully drying and storing this base collection sample at sub-zero temperatures, and using seed from the base collection sample for regenerating every second or third generation for the active collection. Improved technologies and new facilities help insure that these valuable resources will be available in future years with minimum genetic shifts.

Core subsets consisting of about 10% of each crop collection are being developed to represent most of the genetic diversity of each crop species and its relatives. The NPGS is identifying and characterizing a core subset of each major crop to facilitate the use of plant germplasm resources in crop improvement and to improve efficiencies of breeders and active collection curators.

Recent international agreements such as the Convention on Biological Diversity will impact acquisition and exchange of germplasm, but the NPGS's goal is to maintain the free exchange that is needed to continue to increase agricultural productivity to be able to feed the increasing world population in the future.Not only have public and private scientists used introduced germplasm from the NPGS and other sources to produce stress tolerant and high yielding varieties and hybrids, but also farmers have used these improved products to increase yields and lower production costs so that the average U.S. family now spends less than 12% of its income for food.

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