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Genetic Considerations in Germplasm Collection and Maintenance: Introduction to the Symposium

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Agricultural workers in many developed and developing countries are interested in collecting, evaluating, and maintaining germplasm of the crop species used in their particular agricultural production systems. However, opportunities to collect germplasm from centers of origin are being reduced as land is developed for agriculture, roads, housing, and other uses. Of the 240,000 angiosperm species thought to exist currently, it is estimated that 50,000 will become extinct by the year 2000 (2). The urgency to collect accessions of useful or potentially useful plant species is driving many germplasm institutions to expand. Thus, administrators of germplasm institutions are worried about their ability to maintain accessions unchanged for use in solving future problems (1). With such a large effort involved in the areas of germplasm collection and maintenance, it is important to use efficient collection methods, as well as proper means to preserve accessions.

The purpose of this symposium is to describe efficient methods for collecting germplasm from regions of interest, sampling from collections to meet breeding objectives, and preserving accessions so as to minimize unwanted genetic changes. The topics are presented in the following order. First, G. Namkoong describes methodologies for collecting germplasm from the wild. His discussions involve consideration of genetic variability in the populations being sampled as well as the probability of success that the collector requires. E. Ryder then considers sampling from an established germplasm collection to identify accessions needed for a plant breeding program. This discussion is followed by a review of the USDA germplasm system currently used in the United States, presented by P. Fitzgerald.

The second part of the symposium is directed toward proper maintenance of accessions in a collection. E. Roos will cover the important area of genetic changes over time in stored seeds. L. Towill will present a view of techniques for preservation of clonal materials that are important now, or will be in the near future. Such techniques may make it possible to store many clonally propagated accessions without the need to maintain fully grown plants in a clonal nursery. Finally, I summarize all of the areas presented in a brief review of the important considerations for this area of germplasm research.

We in the germplasm and genetics working group of ASHS hope that this symposium will help improve our ability to collect and maintain accessions of species that are useful to crop researchers, and to agriculture in general.

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Sampling for Germplasm Collections

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The person who samples for germplasm collections occupies a very narrow temporal interface between the genetic resources that history has left as our endowment, and the potential uses of those resources with which we endow the future. It is a narrow interface because the genetic resource is eroding rapidly, and because our future needs are large and unpredictable. Given the structural complexity of most plant populations and the many possible ways that they may be used in the future, it is obviously impossible to prescribe simple, optimal sampling schemes for all species. However, a single unstructured sample of modest size would be sufficient for a species that is structured simply. Such a species would have to be structured homogeneously with complete inbreeding or with independently and uniformly distributed alleles in Hardy–Weinberg genotypic frequencies. Future breeders would have to reassemble any allelic combinations. If specific alleles were identified as useful, the needed sample number might be smaller than if the allelic distribution was heterogeneous and unknown in the population and if the potential uses of many such alleles were uncertain. Unfortunately, we are usually in the latter position with respect to what is presently known about frequencies and distributions. We are often ignorant of which alleles are desirable at specific loci. Even for primary grain crops, little is known of which populations to target for sampling and enhancement for future development in breeding programs. For less-studied plant species, it is necessary to consider the biological problem of how alleles are distributed in populations and the kinds of collections that might be useful to future conservators, breeders, or other users. Nevertheless, it is possible to derive some bounds for requisite sample sizes, despite the complex factors that influence sampling design.

Initially, the frequency distribution of alleles at a locus, the distribution of allele frequencies among loci, and the distribution of alleles over populations will be discussed. Several objectives of collecting, including the probability of saving alleles and populations in addition to defining loss-rate functions, before deriving some bounds on sample sizes, will be addressed later. Several important related issues are not discussed, including estimates of minimum viable population sizes required to maintain genetic variability (3) or to permit certain post-collection operations such as selection and breeding.

FACTORS AFFECTING ALLELE DISTRIBUTION

The simplest population models contain no complicating factors, such as linkage, inbreeding, migration, sexual- or age-dependent effects, or subpopulation differentiation. Lacking any information about allelic distribution, it is commonly assumed that alleles may occur at low frequency but are uniformly distributed throughout the population. These models may not represent accurately the majority of biological situations, but they provide a basis for beginning estimates of sample population sizes. For species that are so simply structured, sampling procedures can be outlined.

Considering the distribution of alleles at a locus and assuming the possible existence of many distinct mutants, any finite sample will carry a nonzero probability of missing an allele. A unique allele in a population of size N, exists at a frequency of 1/(2N), and only a complete sample of all N individuals can assure its inclusion. That objective is attained easily in a small population; however, it is not feasible for most species with large populations. Some probability of loss must be accepted. The problem is to determine the sampling cost we are willing to bear for an acceptably low probability of allelic loss. This problem is compounded when loci have several alleles at low frequency, and if we wish to save all of the low-frequency alleles.

Whether these allelic variants exist at these low frequencies due to selection or drift, the conservation objective may be to sample all potentially useful alleles. However, many potentially useful low-frequency alleles might exist, but most unique mutants are expected to be deleterious and of no value to past or future populations. Our interest is in mutants that may now, or at one time, have been favored in the population and therefore exist at some frequency greater than the classical selection–mutation balance. Thus, we may wish to design a sampling system that has a nearly zero probability of missing alleles that exist in the frequency range of 10^{-2} to 10^{-4}. We will then be willing to accept higher loss probabilities for alleles at lower frequencies.

Another major factor that complicates the design of sampling strategies is multiple loci about which little is known. Not only do the number of alleles differ among loci, but they may occur in association with alleles at other loci for reasons that may be of selective significance or merely by chance association. For purposes of rough estimation, consider that 10,000 to 50,000 loci exist, and about one-third of these have allelic variants (5). If as many as one-third of these varying loci carry useful allelic variants, and as many as 10% of these alleles are in the 0.01 to 0.001 frequency range, as can be approximated for neutral loci (2), we may only be concerned with a few hundred loci. In sampling for multiple loci, the probability of saving all alleles is most easily treated as if allelic distributions were independent. The joint probabilities of saving all alleles are decreased when occurrences are negatively correlated. For low-frequency alleles, however, the effect of correlated frequency distributions of alleles at different loci is small, and the approximate sample sizes required are not much larger than those computed for independent loci.

The number of loci as well as the number and frequency of alleles at each locus therefore affect the sample sizes required. In addition, the existence of population subdivisions increases homozygote frequencies over Hardy–Weinberg expectations, increasing the needed sample sizes, since homozygotes contain only one of the alleles. It also increases the need to sample among different areas, sexes, and age classes if differences in allele frequencies are associated with these factors.

Under such conditions, the sampling problem can be stated in terms of the random sample size required to provide a given probability of missing alleles that exist in an array of frequencies. Required sample sizes can be large for many alleles at low frequency at one locus. If we define a minimum allele frequency of concern (α), then the worst possible case is where there are n alleles, all at a frequency of $\alpha = 1/n$. The most stringent requirement for each locus is that no alleles are missed in the sample and, for any given probability level of that occurrence, upper limits on sample sizes can be computed (4). For less-stringent conditions, such as for any small number of alleles at low frequency, the exact probabilities can be computed (7).

The genetic architecture of populations usually is not as simple as in the previous examples. Genotypes generally are not homogeneously distributed in steady-state Hardy–Weinberg frequencies. For species with age structure, reproduction usually is restricted to certain dominant age classes and often to certain dominant individuals. If those dominant individuals repeatedly reproduce during their fecund period, there is an even greater tendency for populations to differ in allelic and genotypic frequencies. If genotypes differ in sexual potency, heterogeneity among groups can increase. It then

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would be important to construct a sample that avoids sampling of related individuals. Unfortunately, finding independent families may be difficult in the field, and the economic pressures for rapid sampling in clusters may lead to sampling of related lineages.

An additional problem caused by such natural, nonrandom allelic distributions is that subpopulation differentiation is easily generated when natural reproduction occurs with few parents and when populations derived with limited numbers of parents are exposed to strong but localized selection. Cryptic population subdivisions have been found to exist in several forest tree species, even when the species appeared to be continuously distributed (8). In fact, separate subpopulations with differences in allele frequencies often exist in plant species. It is usually unclear whether the differences are due to selective factors or merely reflect the fact that individuals within local populations may have only a few recent ancestors. The alleles, therefore, may or may not have some selectively important function or, more importantly, some future significance. Hence, the distribution of samples over population subdivisions affects the probabilities of missing potentially useful alleles. The mating and selective causes of differentiation decrease the frequency of heterozygotes below that expected for completely random mating. Correspondingly, the size and dispersal of samples needed to ensure low probabilities of losing alleles are increased. Only disassortative mating or heterozygote selection increases heterozygote frequencies over Hardy–Weinberg expectations.

COLLECTION SIZES

In a diploid plant, each locus will carry an allele at either 0.0, 0.5, or 1.0 frequency. If the objective is to sample alleles, and not sets of alleles among loci, the size of the seed or pollen sample needed from each plant to save all of its alleles is small. If the frequency is either 0 or 1.0, one seed is as good as any number for saving the allele. However, if the allele is unique and the individual is a heterozygote, then the probability of loss in r gametes is $(0.5)^r$ when sampling either pollen or seed. To ensure a probability of loss of $< 1\%$, $r = 7$ is sufficient. For quantitatively inherited traits, 50 independent loci may affect a trait and, for this number, an r of 13 is sufficient to keep the probability of loss of any of them $< 1\%$. Therefore, gamete samples in the range of 10 to 20 per individual are enough to ensure sampling all alleles. The only reason to collect more seeds would be to sample the pollen population as reflected in the genotype of the maternal parent samples. In sampling asexually propagating plants, the only reason to collect more than one propagule would be if chimeral variations exist, or for safety of the collection; therefore, sampling at the individual level is not a substantial problem.

Sampling genotypes that carry low-frequency alleles in the population is a major problem. For this purpose, it is assumed that n diploid genotypes are independently sampled. The n individuals might be represented by one or a few vegetative propagules of each or by r seed of each genotype. If r is greater than one, then the effective sample size is larger than n by a factor that depends on the mating pattern.

Assume that a single, homogeneous population exists in a steady-state allele frequency distribution with genotypic Hardy–Weinberg frequencies. We can now construct the probabilities of allele loss directly. For a single locus with an allele at frequency q, and a sample of n diploid genotypes, the probability of allele loss is shown in Eq. [1a]:

$$\text{Prob. (loss of an allele at a locus)} = (1 - q)^{2n} = Q, \quad [1a]$$

$$\text{and Prob. (saving the allele)} = 1 - (1 - q)^{2n} = 1 - q. \quad [1b]$$

The effect of departures from Hardy–Weinberg frequencies through inbreeding or population heterogeneity is to increase the Prob. (loss) for a given n, so that the Prob. (save) is diminished. In terms of an inbreeding coefficient, F, we can rewrite Q as:

$$Q = [(1 - q)^2 + Fq(1 - q)]^n \quad [1c]$$

To expand consideration to s loci, and assuming stochastic independence of the rare alleles at each locus, the probability of saving at least one sample of equally rare alleles at each locus is:

$$\text{Prob. (saving at all loci)} = (1 - Q)^s \quad [2]$$

The probability of loss of an allele at at least one locus is greater if the alleles are negatively associated.

To expand consideration to multiple alleles at each of the loci (6), the probability of allele loss in Eq. [1a] must be increased to:

$$\text{Prob. (loss of any allele at a locus)} =$$

$$Q = \sum_{i=1}^{L} (1 = q_i)^{2n} + \sum_{i,j=1}^{L} (1 - q_i - q_j)^{2n} - \sum_{i,j,k=1}^{L} (1 - q_i - q_j - q_k)^{2n} + \cdots + (1 - \sum_{i=1}^{L} q_i)^{2n} \quad [3]$$

Gregorius (4) derives a lower limit for sample sizes needed for the extreme case where the minimal allele frequency is shared by all L alleles, such that the common allele frequency is 1/L. For cases of up to four low-frequency alleles, Namkoong (7) lists requisite sample sizes.

In Tables 1 and 2, sample sizes necessary to ensure low probabilities of missing alleles are given. For each sample site, $(1 - Q)^s$ equals the probability of no losses for sample sizes of n, for the listed number of loci, each with the given rare allele frequency. There is a Bernoulli trial for each of s independent loci, with probability of success of $(1 - Q)$, and hence the expected number of losses is sQ. Therefore, the risks can be expressed as the expected number of alleles lost. Using Tables 1 and 2, an average loss of one allele at one locus can be chosen arbitrarily to be a criterion of an acceptable loss rate.

It is interesting to note that a 10-fold increase in the number of loci considered doubles the number of sampled plants required, whereas a similar decrease in average allele frequency increases the sample size by 10-fold. Thus, sample size is more strongly affected by the rarity of the alleles designated to be saved than by the number of loci. Nevertheless, if many loci affect traits of interest, we may not be as concerned with all of the rarest alleles at each. Hence, sample sizes of several hundred or a few thousand will likely suffice.

Table 1. Minimum number of genotypes required for an average loss of one allele at any of the loci with one rare allele per locus.

No. loci (s)	Frequency of rare allele at each locus			
	0.05	0.01	0.005	0.001
10	45	227	455	2278
50	77	389	780	3906
100	90	458	919	4601

Table 2. Minimum number of genotypes required for an average loss of one allele at any of the loci with four rare alleles per locus.

No. loci (s)	Frequency of rare alleles at each locus			
	0.05	0.01	0.005	0.001
10	72	367	735	3681
50	104	528	1057	5295
100	117	597	1196	5988

Increasing the number of alleles at each locus for 10 loci increases the sample size by ≈62% for all frequencies shown in Table 2. It also can be observed that sample size increase for any increase in numbers of loci is identical regardless of whether one or four alleles are involved. Therefore, the general levels for requisite sample size remain intact.

When sampling a species containing subdivided populations, one approach is to disperse samples into N locations, with n genotypes per sample site (6). If a single allele of interest exists at frequency q in only certain populations, and those populations exist at frequency p, then the Q of Eq. [1a] for a single site sample would be:

$$Q = [(1 - p) + p(1 - q)^{2n}], \text{ and} \qquad [4a]$$

for N site samples, each with n genotypes,

$$Q = [(1 - p) + p(1 - q)^{2n}]^N. \qquad [4b]$$

Any knowledge of patterns of allele dispersal should be used to maximize p by concentrating samples where the alleles may exist or where q may be high. Sampling should be dispersed to include peripheral populations or sites with marginal species fitness in which rare alleles that may be useful in the future are likely to be more frequent. For alleles that are uniformly dispersed throughout the population, p may be close to 1, making any pattern of sampling equally effective. Thus, dispersing samples does not diminish the effectiveness of sampling for homogeneously dispersed alleles, but does increase the effectiveness of sampling the heterogeneously distributed alleles.

The heterogeneity of allelic distributions at a locus thus emphasizes the need for sampling ecological extremes. The more heterogeneous the distribution, the more interpopulation sampling (N) should be emphasized at the expense of intrapopulation sampling (n). The more known of the probable location of rare alleles in the distribution (assuming common alleles are more uniformly dispersed), the more reliance can be placed on cluster sampling within targeted areas. Allard (1) recommends sampling in a regular grid for oats, using 10 seeds/plot, 200 plots per population, five populations per region, and 20 regions per transect, in five transects. Using this sampling procedure, the total number of samples is large, 10^5 maternal plants or 10^6 seeds, but it ensures sampling useful populations.

The independence of allele distributions among loci raises a further question as to whether sampling efficiency can be improved by identifying sites where there is a common occurrence of rare alleles. If there is a positive correlation in the occurrence of rare alleles, then the probability of saving all alleles is increased for a given sample size. However, if sites can be targeted where rare alleles occur, it is obviously more efficient to use that information in sampling. If an objective of germplasm collections is also to save useful populations, perhaps for future enhancement, testing, and breeding, then we may wish to sample a finite number of clusters. If, in addition, favorable alleles occur in groups, then clustered sampling from dispersed or targeted sites would be useful, with sufficient samples per site to ensure their use as populations. Such collections may be expected to contain higher frequencies of useful alleles or allele sets; hence, the sample size per site can be lower than one in which allele distributions are ignored. Other sampling patterns may be required to supplement any targeting patterns; to the extent that there are favorable alleles that lie outside of those clusters.

CONCLUSION

Sample sizes required to ensure low probabilities of losing any alleles, or of losing one or two, are not very large unless the alleles are quite rare. Clearly, the more that is known about present allelic distributions and their future uses, the easier it is to include them in smaller, targeted samples. However, even with substantial ignorance on both accounts, the sample sizes required may range from a few hundred to a few thousand independent, individual genotypes. Problems in defining and sampling "independent" genotypes are present, especially in species with different sexual, age class, or other reproductive and spatial heterogeneities. As a result, dispersed sampling is often needed. Therefore, a significant problem in germplasm conservation may be to secure enough in situ conservation sites to ensure the future continuity of divergent populations or enough ex situ collections to ensure security and availability of the species diversities. Subsequently, the paramount problem becomes maintaining the diversity of these divergent populations in keeping with the overall goal of carrying present diversity into the future.

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Efficient Sampling from a Collection

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The similar titles of Namkoong's paper and this one invite comparison, not so much for their similarity as for the differences in sampling rationale. The main difference is in the nature of the populations that are being sampled. The population in the field is usually far away. Further, it is of unknown size and its genetic makeup and complexity can only be guessed.

Geographical distance means several things. It is expensive to get to a distant place and to stay for a long period. Therefore, the opportunity to sample may not occur very frequently. In some instances, it may happen just once; on a second trip, one may find a city or a cropped field where the collection site used to be.

In the "good old days" of plant exploration, a collector might expect to stay in the field for long periods of time, collect everything of interest in several or many different species, and not worry about costs or whether the sample represented the population genetically and statistically. Now, the collector may have just one short trip in which to obtain a sample of maximum genetic usefulness. Thus, Namkoong presents a set of concerns of real interest to modern collectors regarding the structural complexity of a wild population: its allelic distribution, relative allelic desirability, and other characteristics that would influence decisions on the method of sampling and size of samples.

Sampling from existing collections is a much easier task. Existing collections are easily accessible physically. They are either geographically close or at least communicably close; i.e., questions can be asked and samples requested by telephone or letter. Physical accessibility also means that the collections can be sampled at relatively little expense.

Existing collections have a finite size. Agronomic collections, particularly of the cereal grains, are likely to be quite large and pose sampling efficiency questions that are less likely to arise with horticultural collections, which may be relatively small.

Existing collections should, theoretically, last forever. They are in no danger of being destroyed by urbanization and industrialization, or of being lost because of substitution of improved modern cultivars for land races. However, they can be lost through loss of viability, neglect, or other types of human error or administrative indifference. These are serious concerns that require constant attention.

One of the most important virtues of existing collections is the opportunity to increase the information available from them and, thus, their potential usefulness. To place this idea in proper perspective, it would be useful to consider the present state of existing collections.

It is appropriate that the word "diversity" is applicable to nearly all attributes that describe the state of the various germplasm collections in this country. Unfortunately, at this stage in the development of the national germplasm system, only those collections with some official status can be described adequately. There are many collections that are associated with research projects and are virtually unknown to others. For example, there are several lettuce breeding collections that have no official status and are essentially undescribed.

The size of collections varies tremendously, ranging from small, with a few hundred accessions, to the National Small Grains collection, with 109,000 entries of wheat, barley, rice, oats, rye, *Triticale*, and *Aegilops*. The type of material in the collection contributes to the size variation. Some collections contain only plant introduction lines, some only cultivars, and some only genetic mutant stocks. Some contain combinations of two or all three of these classes of material. The size of the collections is, in general, proportional to the economic importance of the representative crop species but is also related to personal or institutional diligence exerted towards collection and preservation of materials.

The holders of collections are themselves a diverse group. The official national curators include geneticists (tomato and barley genetic stocks), breeders (lettuce, carrot, onion), Regional Station directors (sugarbeet, tomato), and curators *per se* (small grains). They include both ARS/USDA and state experiment station people. There are 29 official curators.

The crops curated include small grains, cotton, sugarbeet, peanut, tomato and tomato genetic stocks, barley genetic stocks, pea, potato, lettuce, *Helianthus*, flax, soybean, pearl millet, *Trifolium*, pecan, tobacco, maize, carrot, onion, native grasses, wheat genetic stocks, Western range grasses, bamboo, tropical fruits, sugarcane, clover, *Tripsacum*, and various woody genera. Official curators have not yet been appointed for most species. Many species are held at the National Seed Storage Laboratory in Fort Collins, Colo., mostly in the form of cultivars. Many are at the four Regional Introduction Stations (Pullman, Wash.; Experiment, Ga: Geneva, N.Y.; and Ames, Iowa) and consist principally of wild and domesticated introductions. Most plant breeders have collections of cultivars and breeding lines and possibly some plant introduction lines and genetic stocks as well.

The diversity among those responsible for the germplasm collections is greatly responsible for the variation in the state of the collections. Many collections are in the hands of plant breeders, geneticists, or other research scientists, whose primary job is research. In those situations, the people, time, and money required for maintenance, evaluation, and distribution are taken from the research projects, with the result that one or another of those efforts suffers from lack of support. This disadvantage is partly offset by the ability of the scientist–curator to translate directly descriptor traits and their values into breeding techniques and improvement. These translations are not always obvious to the curator who is not also in research. On the other hand, I think there is a tendency for a breeder–curator to think of a collection as a personal one, for primary use in the local breeding program, and to neglect the responsibility to describe the material properly and to publicize the existence and availability of the collection.

The lettuce and leafy vegetable collection at the U.S. Agricultural Research Station, Salinas, Calif., is a case in point with regard to some of the previous remarks. It is useful to describe that collection in a little detail. It is a collection I inherited from R.C. Thompson in 1959 and have kept and expanded. In the *Lactuca* group, there are 961 cultivars of lettuce, mostly from the United States, the United Kingdom, and the Netherlands, but also from many other countries. There are 998 plant introduction lines, mostly from countries near the Mediterranean Sea. There are several hundred genetic stocks, consisting of lines with one or more single-gene mutations, as well as early generations from the crosses made for inheritance studies. There are about 2000 breeding lines. Finally, there are miscellaneous accessions consisting of unnumbered introductions, wild material collected from various places in the United States, and other lines. In the *Cichorium* group, there are 277 cultivars of endive and escarole, and various chicory types (witloof, radicchio, and green leafed cultivars). There are also 19 plant introduction lines, nearly all from Western Europe.

The *Lactuca* spp. plant introduction lines consist of 787 entries of *L. sativa*, 138 of *L. serriola*, 13 of *L. saligna*, six of *L. virosa*, and 17 from other species, many of which do not cross with cultivated lettuce. The *Cichorium* spp. plant introduction lines consist of 18 entries of *C. endivia* and one of *C. intybus*.

The only economic character descriptors for *Lactuca* spp. culti-

Table 1. Descriptors for cultivars and plant introduction lines of *Lactuca* spp.

Cultivar descriptors	Plant introduction descriptors
Name	P.I. number (or other)
Date (received or recent increase)	Date of recent increase
Type (crisphead, butterhead, cos, leaf, stem, "Latin")	Species
	Type (*L. sativa* only)
Source—country, organization	Country of origin
Seed color	Seed weight
Leaf color—greenness, anthocyanin	Seed color
Resistances to disease, insect[z]	Leaf dimensions
Synonym	Leaf color
	Leaf shape
	Anthocyanin
	Flowering time
	Flower diameter
	Plant height
	Resistances
	Notes

[z]Lettuce mosaic, big vein, tipburn, sclerotinia, corky root rot; lettuce root aphid.

vars are disease and insect resistance traits (Table 1). Of the diseases and insects listed, only lettuce mosaic virus (LMV) resistance has been evaluated for most cultivars; LMV resistance has been evaluated in our screening program since 1959. There is extensive information published on downy mildew resistance in cultivars, particularly of the butterhead type, but little of this information is yet in our descriptor listing. There is published information on resistance to root aphid, corky root rot, cabbage looper, sclerotinia, and tipburn, but very little has yet been entered into our lists.

Other economic characters—bolting resistance, head development characters, maturity time, yielding ability, and head size—have never been systematically or comprehensively rated either objectively or subjectively and, consequently, little information is available for listing. A newly formed Leafy Vegetable Crop Advisory Committee is beginning to prepare a list with this information. The *Lactuca* spp. plant introduction lines have more information listed for both objective and measurement traits (Table 1). The *Cichorium* spp. collection is new, and little information is listed.

The collections are held in household freezers, which provide satisfactory storage and greater longevity than the more common storage of 0 to 10°C and 40% to 50% RH. However, the containers are crowded and not readily accessible. Most of the seed is in paper envelopes in plastic freezer containers.

Lettuce and *Lactuca* spp. collections are found in other U.S. and European locations (Table 2). Conditions of storage vary. The largest collections listed are those at Wageningen, Wellesbourne, and Leningrad; most others are quite small.

In response to questions about the state of their collections, curators of several other species have indicated that there is a great deal of variability in collection size, types of materials in the collections, and the number of entries evaluated for each of the descriptors listed. It is apparent that those who wish to sample from the collections make very specific requests when they know what they want, and vague requests when they do not. In the latter case, the requestor is usually dealing with a newly arisen problem or is unfamiliar with the species requested.

Table 2. *Lactuca* spp. collections at other locations in the United States and other countries.

United States
Regional Plant Introduction Station, Pullman, Wash.
National Seed Storage Laboratory, Fort Collins, Colo.
Breeding collections
Univ. of California, Davis.
Cornell Univ., Geneva, N.Y.
Univ. of Florida, Belle Glade.
Outside the United States
Research Institute of Plant Protection, Prague, Czechoslovakia
Central Inst. for Genetics and Crop Plant Breeding, Gatersleben, G.D.R.
Institute for Horticultural Plant Breeding, Wageningen, Netherlands
Vavilov All-Union Inst. of Plant Industry, Leningrad, USSR
National Vegetable Research Station, Wellesbourne, U.K.
National Institute for Agricultural Variety Testing, Tapioszeli, Hungary
Germplasm Institute, Bari, Italy

[z]From ref. 1.

How, then, do we make our germplasm collections most informative and most useful? The first and most obvious step is to make the collections themselves grow. The more material there is in captivity, the greater is the potential for its usefulness. The capacity of a collection to provide information and raw material for crop improvement is limited to the extent that the size of the collection itself is limited. Very simply, then, we must collect as many materials as possible.

The traditional means is to collect materials in the crop species and in related species by traveling to centers of origin and development. This is expensive, time-consuming, and often physically difficult, but necessary. Many populations are in transition, i.e., they are often here today and gone tomorrow, and it is imperative that we maintain and even increase the pace of collection.

I have what may be an unusual suggestion for increasing our germplasm base in another way. Plant breeders throw away 90% to 99% of the material they observe because most recombinants from a cross are unsuitable in terms of the goals of the breeding program. On the other hand, it is possible that some of the genotypes may have future economic value. Consumer tastes change. Plant architecture changes may become necessary as tillage methods change. Early or late maturity may become more desirable than current needs dictate. Other characteristics may have to be altered in response either to increased knowledge of physiologic–genetic responses, or to economically or socially inspired outside requirements. Therefore, it would seem useful for plant breeders to save a random or selected sample of segregating populations in addition to the material selected for continued breeding. Seeds of the potentially useful material then could be kept in storage or forwarded to the appropriate curator or other collection holder.

It is necessary to stress the importance of domesticated material, including cultivars and breeding lines, as germplasm. Germplasm often is taken to mean only plant introduction material, and there is a strong implication that its usefulness is in supplying single genes for resistance to save a crop species from a certain disaster brought on by some disease or insect epidemic. This is the biotechnologist's germplasm; the plant breeder's germplasm also includes a vast array of genetic combinations that must be assessed for their usefulness in an equally vast array of environments.

Efficiency in sampling the collections is dependent on several factors. First, the collections must be permanent, i.e., they must be stored in an adequate facility, they must be secure, and they must be monitored for viability and renewed at appropriate times.

Second, collections must be evaluated for both noneconomic and economic traits, a massive job that requires support independent of the associated breeding or other research programs. A number of crop advisory committees delegate responsibility to appropriate researchers to perform specific evaluations: screen for resistance to a disease or insect, measure response to a particular stress condition, etc. This practice should be encouraged. It is relatively easy to do this for crops such as wheat and cotton, where there is a fairly large group of researchers to whom tasks may be assigned. It is more difficult with many horticultural crops, where the number of people associated with the crops may be relatively small.

Third, collections must be publicized so that researchers are aware of their existence, the kinds of information available about the entries, and the fact that the materials are obtainable. I believe that collections should be described and published by the holder as well as being placed in the Germplasm Resources Information Network (GRIN) system. This adds a measure of redundancy of information available, which I consider to be desirable for maximizing awareness of the existence and usefulness of the collection.

Finally, redundancy in the collections themselves is desirable. Any single entry for a species should exist in more than one location. Losses of single entries or sometimes catastrophic losses of entire collections or parts of collections can occur. Redundancy can

prevent irretrievable losses.

Present practices in sampling are based upon the knowledge about and accessibility of the collections, the information available about the entries, and the purpose for which samples are needed. According to the curators, most or nearly all requests are specific, i.e., for a single item or for a group known to have a desired trait or traits. A substantial number of requests are somewhat vague; for example, the requestor may ask the curator to choose material that might be resistant to a disease. There are also many requests for a representative geographic distribution of materials. Rarely are requests for an entire collection or for a whole section of a collection.

One may hope that, in the future, efficiency of sampling will be improved. As the number of traits described increases and as the completeness of description of each trait increases, the greater will be the opportunity for a researcher to request specific items and the lesser the need for vague or shotgun requests. Along with the increases in information will be the greater accessibility of that information by inclusion in the GRIN system. This sort of information development will be most useful for known traits, and will enable the breeders to incorporate into their programs additional useful genes and gene systems for yield, resistance, earliness, etc. It will also provide the geneticist, the physiologist, the plant pathologist, the entomologist, etc. specified, identified materials for appropriate experiments.

It is well to keep in mind, however, that when the research must deal with a new, previously unknown disease or situation for which descriptors do not exist, little will be different. Then, as now, the technique of sampling will depend on the nature and urgency of the problem, the resources available for research, the perception of the researcher and the curator in determining the materials most likely to be useful in solving the problem, as well as other nonobjective considerations.

It is hoped that objective criteria for sampling will be more readily defined than at present. These criteria might include protein analysis of small seed samples to gain clues associated with possible disease resistance, development of probability parameters to estimate sample sizes needed to discover genes for resistance or other traits and availability of and knowledge about genes from other species that may be transferred via new technology to the species of interest. Whatever the future holds, there will be no substitute for a well-financed system of collection, maintenance, evaluation, and distribution of germplasm in its most broadly defined sense.

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Genetic Considerations in the Collection and Maintenance of Germplasm

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Plant germplasm has become a topic of interest and concern at the highest levels of our government and in many other parts of our society. Part of this concern no doubt relates to the increased awareness by leaders in the government and in the public at large that U.S. agricultural productivity and stability is dependent on plant resources that are not native to the United States. The United States, as compared to many agricultural producers in the world, has no centers of origin for important food and fiber crops. In fact, the United States has no major crop native to its geographical area. Of all the crops important to U.S. agriculture, only sunflower (*Helianthus* spp.), Jerusalem artichoke (*Helianthus tuberosus* L.), grape (*Vitis* spp.), pecan [*Carya illinoensis* (Wangenh.) C. Koch] and other hickories (*Carya* spp.), blueberry (*Vaccinium* spp.), cranberry (*Vaccinium macrocarpon* Aiton), and a few other miscellaneous species of minor importance are native to the United States.

Human beings have displayed an interest in plant germplasm throughout recorded history, and probably for a long time before. The involvement of Alvin Toffler led him, in a 1984 television special, to say "10,000 years ago some genius reached out and altered our lives forever . . . by planting a seed". We are beginning to appreciate the essential role plant germplasm has played in the development of all societies throughout history and, perhaps more important, in its essential role in our future.

Our earliest understanding of the importance of germplasm began with the first seed savers. These primitive agriculturists developed a practice of collecting seed from chosen plants for the next crop and, in so doing, unknowingly became the first to engage in activities later to become a part of plant breeding. The scientific basis for understanding, however, was not to come until about 1900, when Gregor Mendel's principles of heredity were rediscovered and provided evidence that characteristics of all organisms are passed from one generation to the next in an orderly and predictable manner, controlled by genetic factors, later called genes. With this new knowledge of plant behavior, improvement of important crop plants became an attainable goal of plant breeders and program leaders. These efforts to manipulate genes to produce better plants have been one long progression from observation to explanation, from art to science.

The recognition of the value of specific genes led to other conclusions. Continued improvement of plants—those for food, feed, fiber, industrial uses, and research—is dependent on a continuing and adequate supply of germplasm with useful genes. A major supply of needed genes will have to be provided by accessions (seed samples) held in storage in various crop seed collections and by plants in clonal germplasm repositories.

Adequacy of the collections to provide useful genes for current and future programs will be dependent on how successful collecting efforts have been in obtaining good representation of the existing genetic diversity within crop species and close relatives and on the quality of preservation procedures and facilities. Collectively, genes within a crop species, or a group of related species, represent genetic diversity with which scientists must work. It should be the goal of all collections to preserve the highest level of useful genetic diversity that is feasible. Further, to be most useful, the genes collected must be readily available to those who wish to use them in plant breeding and research; otherwise, progress in crop improvement may be halted. Availability is linked to identification and evaluation, and it is in this area that much additional work is needed.

Plant breeders were probably the first to recognize the need and to take action to gather, for themselves, a collection of plants containing potentially useful genes for later use. These efforts led to the awareness that special handling and facilities were needed to preserve the germplasm samples, usually seed, until needed. The periodic requirement to grow the samples in a protected nursery to multiply the seed and to make preliminary observations and evaluations of the plants also was recognized as necessary to maintain seed collections with acceptable viability and genetic reproducibility. Further, it also was recognized that some valuable crop germplasm, such as fruit and nut species, could not be suitably preserved as seeds. Consequently, a system of clonal germplasm repositories was initiated to protect and preserve, as plantlets or whole plants, the most important fruit and nut species. A national

Table 1. National clonal germplasm repositories for fruit and nut species.

1) National Clonal Germplasm Repository, Corvallis, Ore.
   Hazelnuts, pears, small fruits
2) National Clonal Germplasm Repository, Davis, Calif.
   Grapes, stone fruits, walnut, pistachio, fig, olive, kiwi, persimmon, mulberry, pomegranate
3) National Clonal Germplasm Repository, Geneva, N.Y.
   Apples, American grapes
4) National Clonal Germplasm Repository, Riverside, Calif.
   Citrus, date
5) National Clonal Germplasm Repository, Orlando, Fla.
   Citrus
6) National Clonal Germplasm Repository, Brownwood, Texas
   Pecans, hickories, chestnuts
7) National Clonal Germplasm Repository, Hilo, Hawaii
   Macadamia, pineapple, guava, papaya, passionfruit, breadfruit, cashew, acerola cherry
8) National Clonal Germplasm Repository, Miami, Fla., Mayaguez, Puerto Rico
   Avocado, banana, mango, Brazilnut, coffee jujube

plan was developed to establish 12 clonal repositories. Later, this number was reduced to the current 8 repositories (Table 1). The first clonal repository was dedicated in 1981 at Corvallis, Ore.; the second at Davis, Calif. in 1984; and the third in Geneva, N.Y. in 1986. All are expected to be completed by early 1988.

The acquisition of germplasm began with the first settlers to this continent as they brought seeds to their new homes from wherever they came. These efforts continued throughout the 18th, 19th, and 20th centuries as diplomats, travelers, and explorers were urged by the U.S. government to collect and send home seeds of interest and agricultural importance. These activities became better organized after the USDA created the Section of Seed and Plant Introduction in 1898. This office was headed by David Fairchild (1), who later hired Frank Meyer (2) and sent him on three plant explorations to China and one to central Russia. Meyer was an outstanding botanist and possessed unusual energy and zeal to collect plants of agricultural value to the United States. We are greatly indebted to his untiring efforts. His collections numbered in the thousands and cut across many of our field, vegetable, fruit, nut, and ornamental crops. Many of his plants were first grown at the USDA Chico Station in California.

Recognition of the scarcity of native crop plants in the United States provided a strong impetus for collection of a wide range of germplasm from all parts of the world, and this effort was reinforced by the passage of the Research and Marketing Act in 1946, which established a National Cooperative Program to link the efforts of the federal and state governments in the preservation of germplasm. Out of this Act came the authorization to establish Regional Plant Introduction Stations. Four stations were established between 1948 and 1953—at Ames, Iowa; Experiment, Ga.; Pullman, Wash.; and Geneva, N.Y. Construction of the National Seed Storage Laboratory at Fort Collins, Colo. followed in 1958. This facility is almost filled to capacity and is in great need of expansion and upgrading, as are many of the others.

The National Plant Germplasm System (NPGS) and the National Plant Germplasm Committee (NPGC) have evolved over a period of several years, working in harmony and cooperatively with the International Germplasm System led by the International Board of Plant Genetic Resources. The NPGS is a coordinated and cooperative effort by the public and private sectors, universities, research organizations, and individuals to collect, catalog, maintain, evaluate, enhance, distribute, and preserve plant germplasm of value and interest to U.S. agriculture. The components of the system are guided by a common objective and linked together by the Germplasm Resources Information Network (GRIN), a computerized data management system. The efforts of GRIN are coordinated by the NPGC, made up of representatives from the federal government, state institutions, and industry, with observers from Canada and Mexico, ASHS, and the Crop Science Society of America. The NPGS and NPGC rely heavily on advice and recommendations from more than 35 crop advisory committees made up of key scientists and crop specialists representing the various commodities. Other sources of information are used as well to operate the system.

The NPGS is designed to provide, on a continuing, long-term basis, the plant genetic diversity needed by public and private scientists and farmers to improve productivity of crops and minimize the vulnerability of those crops to biological and environmental stresses. The system recognizes the need to accommodate and use advances in biotechnology in order to take full advantage of existing genetic diversity. New genetic tools that are becoming available have increased interest in germplasm collections as sources of genetic variation necessary for exploitation of this advanced technology to improve plants. The capability to identify, locate, and move genetic material between plants not closely related will expand the opportunity to more freely use the gene pools represented by existing U.S. collections. New scientific developments in molecular and cell biology, such as gene sequencing, may eventually prove to be the most reliable method to describe germplasm characteristics. At the same time, the new capabilities to manipulate genetic material provide strong justification to reassess existing base collections and ensure that each adequately represents the genetic diversity available in other collections and in nature. These new capabilities certainly suggest that more attention will need to be given to collection and preservation of primitive types and wild relatives of existing crop plants. A major objective of the NPGS should be to bring all U.S. collections to a level of self-sufficiency in its germplasm resource base. Where valuable germplasm is in danger of being lost or becoming unavailable, immediate attention should be given to further germplasm exchanges and exploration. In most instances, evaluation of existing collections will suggest what additional germplasm should be added to the collections to fill the gaps.

Others in this symposium report on research directed to a better understanding of the collection, maintenance, and preservation of germplasm. This kind of research is urgently needed to expand our knowledge on more efficient and less costly ways to maintain genetic diversity that is both representative and adequate for long-term crop improvement and production. This and related research on evaluation and enhancement merits recognition and support at all levels of government.

In the long-term view of the future, the highest priority for any nation must be to provide and protect the means for its citizens to feed, clothe, and shelter themselves. Continued crop improvement will be necessary to maintain production sufficient to the needs of a growing world population. Future crop cultivars will have to produce more per unit area and do it more efficiently and economically than in the past if acceptable standards of living are to be maintained. All crop improvement is dependent on available genetic diversity in nature or in our collections. It is essential that representative and adequate samples of this genetic diversity be preserved for the present and future generations. Much has already been accomplished, but much remains to be done.

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Genetic Changes in a Collection over Time

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Much emphasis has been placed on the need to preserve plant genetic resources (13, 17, 18, 28, 31, 50). As a reuslt, the number of accessions added to gene banks around the world has risen dramatically over the past 10 to 12 years. The International Board for Plant Genetic Resources (IBPGR), during its first decade of work (1974–84), arranged for the collection of ≈121,000 samples of germplasm from more than 90 countries around the world (19). At the USDA National Seed Storage Laboratory, accessions in storage have increased from 91,000 in 1976 to more than 204,000 in 1986. These accessions represent ≈370 genera and 1960 species. The large influx of samples during recent years has placed greater responsibility on germplasm curators to ensure that samples are properly handled, including periodic germination testing and regrowing of samples when needed.

Germplasm preservation strategy

Two strategies for preserving germplasm are in situ conservation in natural preserves and ex situ preservation, usually in gene banks. Although in situ preservation allows for continued evolution of a species, large areas of land are needed and species are still subject to genetic wipe-out as a result of natural disasters such as fire, earthquake, flood, or even volcanic eruption. Preservation ex situ is thus the preferred method for genetic preservation in those species where seed storage presents relatively few problems. Having stored the samples, these must be monitored periodically for viability and new samples must be regenerated when viability declines or seed numbers become low (Fig. 1). It is the storage and regeneration phases of germplasm maintenance with which I will be concerned in this paper.

Types of genetic changes

Genetic changes fall into two categories: a) Spontaneous genetic changes, such as point mutations and chromosomal aberrations; and b) genetic shifts, such as changes in gene frequencies or loss of genes from the population sample. De Vries (8) has been credited by some authors (22, 36) with being one of the first researchers to recognize the relationship between seed aging and genetic changes. However, Priestley (34) has argued convincingly that these data have been misconstrued and are probably irrelevant to the question of spontaneous genetic changes in aged seeds. Nawaschin (29) and Peto (33) are credited with the discovery of the relationship between seed deterioration and spontaneous genetic changes. Population shifts in germplasm collections have been discussed much less than mutations, although they can seriously erode genetic diversity in a collection undergoing frequent seed regeneration (43).

SPONTANEOUS GENETIC CHANGES

Research on spontaneous genetic changes has focused primarily on production of chromosomal aberrations in root tip cells from aged seeds that are undergoing the first mitotic divisions after initiation of germination. Various reviews have concentrated on the production of aberrations in both artificially and naturally aged seeds and the relationship between germination percentage and frequency of aberrations (3, 4, 7, 36, 37, 40, 41). With the exception of seeds artificially aged under extreme conditions (storage temperature above 40°C and seed moisture content ≥18%), it appears that seed aging results in a negative correlation of germination percentage with percentage of chromosomal aberrations in seedling root tips. One problem associated with these studies is the timing of first mitosis, particularly in aged seeds. Murata et al. (24) showed that seed aging both delayed mitosis and reduced mitotic indices in root tip cells of peas. In later studies, this delaying factor was taken into consideration in the detailed analysis of mitotic configurations in aged barley seeds (26).

Point mutations have received less attention than chromosomal aberrations, probably because of the large numbers of seedlings or plants that must be examined, and the necessity of following the changes through one or two generations in order to demonstrate that they are, indeed, genetic mutations. However, as discussed below, point mutations may have a more lasting effect on germplasm than chromosomal aberrations.

Chromosomal aberrations

Results of the many studies on chromosomal aberrations occurring in aged seeds lead to the following three important conclusions. The first is that there is a negative correlation between seed germination percentage and percentage of aberrant root tip cells (Fig. 2). Studies by Abdalla and Roberts (1) and Murata et al. (25) clearly demonstrate this relationship. This correlation appears to hold for several genera, including *Antirrhinum, Cannabis, Datura, Nicotiana, Nothoscordum, Solanum* and probably many others (41), and for a fairly wide range of storage conditions. As previously mentioned, there are some exceptions, however. For example, broad bean (*Vicia faba* L.) seeds stored at 45°C and 18% seed moisture content (high-stress conditions) showed only a slight increase in aberrations as compared with seeds stored at 25° or 35° and 18% moisture content (36). Under high-stress storage, seeds appear to die rapidly, so that abnormal seedlings are not detected. Recently, Hang et al. (14) examined different storage temperatures, including that of liquid N (−196°), to determine if chromosomal aberrations might be induced by extreme cold. No loss in germination occurred at this temperature, and they were unable to detect any induced chromosomal aberrations.

The second conclusion holds that chromosomal aberrations occurring as a result of seed aging are not transmitted to the next generation. From a germplasm curator's perspective, this element is critical in the long-term maintenance of the genetic integrity of an accession or a collection. Elimination of chromosomal aberrations appears to begin almost immediately after root tip growth is initiated. Nichols (30) reported a decline in percentage aberrations

PRESERVATION OF GERMPLASM

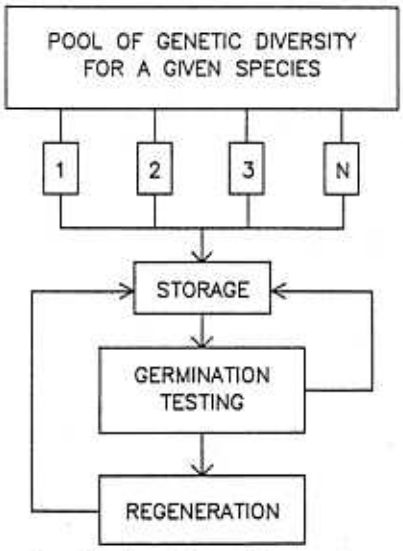

Fig. 1. Strategy for collecting and preserving germplasm accessions.

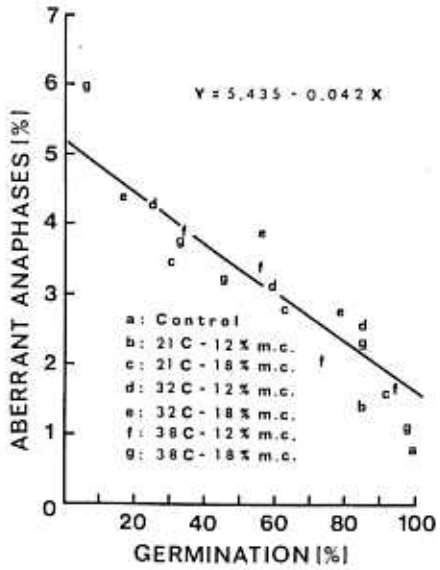

Fig. 2. Relationship between the frequency of aberrant anaphases in barley root tips and percent germination. Data from Murata et al. (25).

from 10.4% to 1.7% in root tip cells of onion (*Allium cepa* L.) as root length increased from 2 to 5 mm to 80 to 100 mm. Peto (33) subjected barley (*Hordeum vulgare* L.) seeds to heat treatment, which reduced germination from 99% to 67%. In young seminal root tips, the frequency of aberrant cells was 27%, and declined to only 9% in root tips examined 40 days after seeding. Similar results have been reported by D'Amato (6) and Kato (21). In aged lettuce (*Lactuca sativa* L.) seeds, chromosomal aberrations observed at first mitosis were not transmitted to the next generation (15, 16). Murata et al. (27) made an extensive survey of the fate of aberrations in aged (A_1 generation) barley seeds. They recorded the frequency of aberrations at first mitosis and after 3 and 5 weeks of growth in root tips. Meiotic aberrations were recorded at first and second anaphase and at the tetrad stage in pollen mother cells. Finally, first mitotic anaphase cells were examined in root tips of the next (A_2) generation of seeds. The frequency of aberrations in plants grown from aged seeds declined during plant growth and reproduction and was indistinguishable from that in unaged seeds in the A_2 generation (Fig. 3). From these results, it can be concluded that chromosomal aberrations induced by aging are not transmitted to the next generation.

The third conclusion is that the mechanism for production of aberrations most probably involves DNA synthesis and/or repair and not simply chromosome breakage. Chromosome aberrations can be designated as chromosome-type or chromatid-type (45). The chromosome-type aberrations involving entire chromosomes are known to result from effects on chromosomes prior to duplication (i.e., during the resting stage prior to seed germination). Aberrations involving chromatids are thought to ensue from effects on duplicated chromosomes and would indicate that the chromosomal damage is induced after resumption of cell division during the seed germination process.

Authors have disagreed on whether chromosome or chromatid-type aberrations occur in root tips from aged seeds (9, 41). A detailed study of 767 anaphase configurations of root tip cells from aged barley seeds resulted in a classification of structural changes as fragments, bridges, or combinations of bridges and fragments (Table 1) (26). Almost 44% of the cells contained single bridges. According to Lea (23), single bridges, not accompanied by one or two fragments or rings, do not occur as a consequence of either a chromosome or a chromatid break. Evans (12) classifies these as subchromatid exchanges that result in bridges because "coiling of the chromatids frequently interferes with the separation of the exchanged regions." The second most frequent class involved cells containing single fragments, which classically would be interpreted as occurring due to chromatid-type aberrations. The third most frequent class was double bridges, again with no fragments, indicating that these probably did not arise from chromosome-type aberrations. Thus, <25% of the aberrant anaphase cells recorded came from chromosome-type aberrations; results that essentially were duplicated by Dourado and Roberts (9). These data suggest that actual damage to cells did not occur until after the start of germination and, as proposed by Villiers (47, 48) and Villiers and Edgcumbe (49), may involve a mechanism in which the natural repair processes of the cell are the target of damage from seed aging. In contrast, Dourado and Roberts (9) have argued that aberrations arise as a result of initial lesions in the DNA that only become visible after replication and, therefore, occur during the seed storage period. Although there is controversy about the origin of aberrations, most

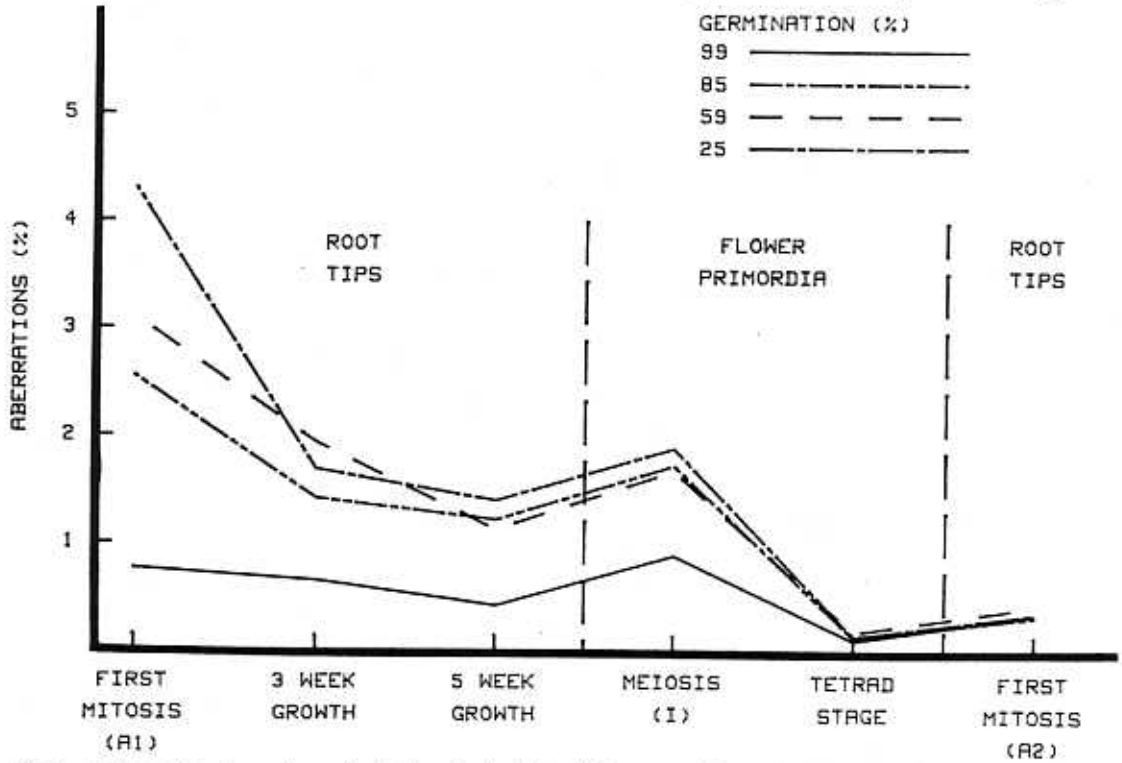

Fig. 3. Effects of plant growth on elimination of aging-induced chromosomal aberrations in barley seeds. Data from Roos (41).

Table 1. Types and proportions of chromosomal aberrations observed at first mitosis in artificially aged barley seeds. Data from Murata et al. (26)

Type of aberration	Aberrations	
	No.	%
1) Single bridge	336	43.81
2) Single fragment	141	18.38
3) Double bridge	113	14.73
4) Multiple bridge	49	6.39
5) Double fragment	45	5.87
6) Single fragment and single bridge	28	3.65
7) Multiple fragment	17	2.22
8) Double fragment and single bridge	12	1.56
9) Single fragment and double bridge	2	0.26
10) Double fragment and double bridge	2	0.26
11) Others	22	2.87
Total	767	100

authors agree that the mechanism(s) of seed deterioration probably involve(s), to some extent, disruption of the normal DNA replication cycle, which, in turn, affects cell division and subsequent viability of the daughter cells.

Point mutations

Detecting point mutations as a result of seed aging poses a more difficult problem for the following reasons: a) large numbers of seeds must be evaluated; b) recessive mutations may not appear until the F_2 or, in some cases, the F_3 generation; c) many point mutations can only be detected biochemically or by means of complicated genetic analyses. Also, many morphological abnormalities that are not inherited may arise as a result of seed aging.

Relatively few studies have been conducted on induction of point mutations as a result of seed aging. Abdalla and Roberts (2) aged seeds of barley, broad beans, and peas (*Pisum sativum* L.) at different combinations of elevated temperature and seed moisture content to reduce germination to ≈50%. This treatment resulted in nonheritable morphological abnormalities in 10% of the bean plants, 5% of the pea plants, and none of the barley plants. There was a significant increase in chlorophyll-deficient mutations in all species, as well as increased pollen abortion. In a later study (10, 11), barley and pea seeds were examined in the second and third generation after seed aging to confirm the induction of chlorophyll-deficient and other mutations as a result of seed aging.

Although a case can be made for the induction, inheritance, and possible accumulation of point mutations in seed accessions as a direct result of seed deterioration, it is my opinion that the net effect of these mutations on a germplasm collection is probably of minor significance as compared with the effects of genetic shifts. It is well-known that most mutations are deleterious and are quickly eliminated through natural selection. Also, even in seed lots where viability has declined 50% or more, the percentage of mutations is only on the order of 1% to 4% (10, 11). There is also the possibility that mutations could be beneficial, or at least serve as genetic markers and thus be of value in expanding the genetic variability within a germplasm collection. For this reason, there is little need for concern about mutation as a significant factor in altering the composition of germplasm collections.

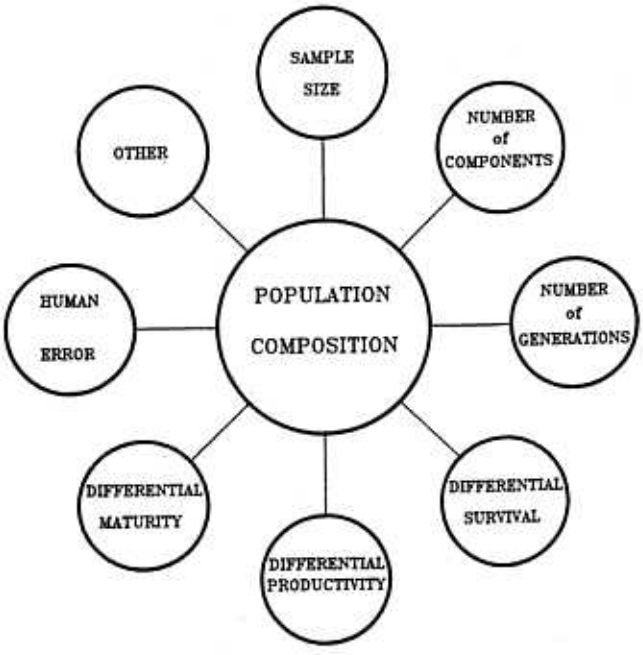

Fig. 4. Factors affecting population composition of a heterogeneous germplasm accession.

GENETIC SHIFTS

Collection and maintenance of germplasm samples necessitates the genetic study of small populations. Although accessions may be self-pollinating or cross-pollinating, most are heterogeneous mixtures (42). The reason for this heterogeneity is that germplasm samples have been collected from natural environments with the goal to collect maximum genetic variability. Assuming that a germplasm accession represents a population, it is desirable to understand factors that alter the genetic composition of the population in germplasm acquisition, storage, and regeneration.

Factors affecting population composition

Several factors have been identified that can affect population composition (Fig. 4). The initial composition (number and frequency of each component) is subject to change due to sampling and frequency of regeneration. Differential survival during storage can result in selection for some components. Productivity and maturity differences in the regeneration process can shift the genetic composition. Human errors such as mixing or mislabeling can result in genetic alteration of a population or collection of germplasm accessions. Other factors, such as disease epidemics, insect attacks, or adverse weather conditions can effect changes in the composition of the population. In this paper, I will only be concerned with the effects of sampling, storage, and regeneration.

Effects of sampling

One of the most important factors contributing to a genetic shift in a germplasm population is size of the seed sample planted for field increase after deterioration in storage or depletion of the accession. The chances of losing a component through sampling a population can be calculated by complex formulas from basic probability theory (32). For example, the probability of obtaining at least one seed of each component in a population containing equal numbers of seeds of 8 components would be 0.31 if only 16 random seeds were taken (39). If 32 seeds were taken, the probability increases to 0.89, and, if 64 seeds were sampled, the probability is 0.99. Repeated sampling of the population (15 times) gives probabilities of 2×10^{-8}, 0.18, and 0.98 for sample sizes of 16, 32, and 64 seeds, respectively. In every instance, equal representation of each component is assumed. However, this would seldom be the situation in an actual germplasm accession.

Effects of storage

Successful seed storage depends on effective control of several factors, including temperature, seed moisture content, and storage atmosphere (44), and variability in response to storage conditions (20, 35, 38). Thus, it should not be unreasonable to expect seeds within heterogeneous germplasm accessions to deteriorate at different rates, thereby causing selection within the sample to favor genotypes more amenable to given storage conditions.

Selection within a germplasm accession during storage, coupled with use of a small sample for seed regeneration, has a powerful influence on the genetic composition of an accession and can eliminate components quickly, particularly those present initially at a low frequency. Roos (42) conducted an experiment on eight cultivars of snap beans (*Phaseolus vulgaris* L.) to model the effects of the seed aging process on the composition of an accession. Cultivars were chosen for differences in either seed coat color or pod color to facilitate monitoring of genetic changes. In this study, artificial seed aging (24) was used to reduce germination percentage and develop survival curves for each component in the mixture. When overall germination reached 50%, relative survival values for each cultivar were calculated and used in a computer simulation program to determine which cultivars would be eliminated and how many generations (cycles of aging) would be required. After 11 cycles using a sample size of 64 (eight seeds per cultivar initially), one-half of the cultivars were eliminated, thereby removing one-half of the genetic variability within the population. In addition, two of the remaining cultivars comprised only five seeds each in a 64-seed population.

Selection during regeneration

Whereas storage effects are manifested primarily through seedling survival (germination), the effects of selection during regeneration can come about through a variety of causes. Differences in productivity of different genotypes increase or decrease the number of seeds of that genotype in the next generation. As mentioned previously, the presence or absence of various selection pressures (disease, insects, weather) greatly affect the productivity of genotypes in an accession. Harvesting a field early or late can result in a selection for different maturities within an accession. In addition, there is always the possibility for contamination, either through cross-pollination or through mixups during the harvesting operation.

In a continuation of the study of the mixed bean population (42), Roos (43) modeled the effects of seed selection during regeneration. Yield capacity of the eight cultivars was determined over a 3-year period, and data were entered into a computer simulation program. After 15 cycles of regeneration (again, one-half of the cultivars were eliminated from the population) solely as a result of differences in productivity (measured as seed yield/plant). However, these cultivars were not necessarily the same four cultivars eliminated by storage treatments. When effects of seed storage and regeneration are combined (Fig. 5), the net result is the total elimination of six of the eight cultivars, with one of the surviving cultivars comprising only seven seeds out of a 64-seed population.

These experimental results demonstrate the potential for loss in genetic variability within heterogeneous germplasm accessions during the seed preservation (storage and regeneration) process. Compared to the spontaneous genetic changes discussed previously, it is apparent that genetic shifts pose a much greater threat to the genetic integrity of an accession or collection.

MINIMIZING GENETIC CHANGES IN A GERMPLASM COLLECTION

From the preceding discussion, it can be concluded that loss in seed viability is the primary factor for genetic changes in germplasm accessions. Therefore, an obvious answer to the problem of minimizing genetic changes in a germplasm collection is to increase the storage life of the seeds. Present recommended standards for long-term storage of germplasm accessions as seeds specify drying to ≈5% moisture content, hermetically sealing the samples, and storing them at −20°C (5). Unfortunately, many seed storage facilities cannot achieve these standards, especially low temperature. Drying seeds, particularly in the humid tropics, presents a significant challenge in many genebanks.

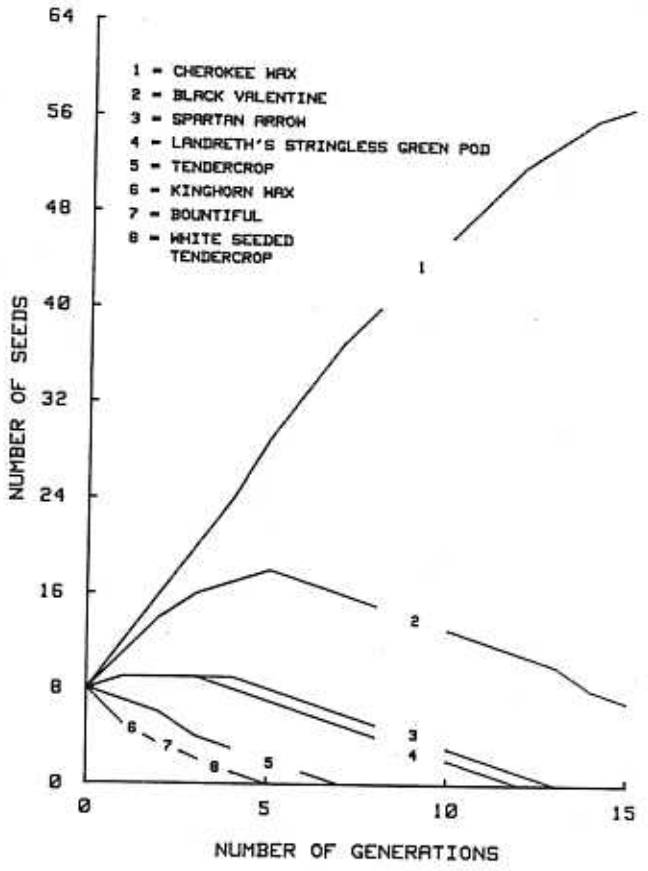

Fig. 5. Simulation of genetic shift in composition of a mixed snap bean population following several generations of seed aging (to 50% population germination) and seed increase (population size = 64 seeds). Data from Roos (43).

Considerable interest has been generated in lowering the storage temperature to that of liquid N (−196°C) (46). There is potential that viability may be prolonged almost indefinitely under this storage condition. At present, more information is needed on the reaction of specific plant species to exposure to these cryogenic temperatures. However, storage in liquid N may be economically justified for small-seeded speices that are notably "poor storers" under conventional storage conditions and are expensive to produce.

Small sample size is another important factor contributing to genetic changes in germplasm accessions, especially during seed regeneration. Small samples cause an increased loss of components in a mixed population through random selection. Doubling the number of seeds used for regeneration (from 50 to 100 seeds) can greatly improve the maintenance of genotypes (or genes) in a population.

SUMMARY

Genetic changes can occur in germplasm accessions during seed preservation procedures. Chromosome aberrations and point mutations have been studied in some detail and have been long associated with loss of seed viability. However, chromosome aberrations are eliminated in most instances by the failure of aberrant cells to divide normally and be included in the next generation of seeds. Point mutations may, in fact, increase genetic variability in a collection, which presents no significant problem to genetic resource managers. Genetic shifts coupled with the use of small sample size can erode genetic variability rapidly within an accession or collection. Germplasm curators must use constant vigilance to avoid these shifts.

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Genetic Considerations for Germplasm Preservation of Clonal Materials

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The present diversity of plants used for the needs of society is immense and could expand greatly with the development of new crops and more extensive use of plants producing useful pharmacological compounds. We are relatively familiar with problems associated with germplasm preservation of major food crops, but such information is lacking for many minor crops. Aspects of genetics, physiology, and phenology must be considered to develop a useful and efficient means of germplasm preservation (29).

Elite clones have been used in agriculture for many years, and preservation is essential for continued development of new cultivars with improved characteristics. Two major considerations for preservation of clonal materials are genetic stability of the line and application of maintenance procedures to diverse arrays of genotypes. Problems with genotypic specificity can occur at any stage of the preservation process, and dealing with this is crucial to establishment of an efficient, usable system.

The manner in which clonal germplasm is preserved is examined here, especially in light of developments in plant microculture and cryobiology. Adequacy of the genetic base is also an important issue, but will not be addressed.

ACTIVE AND BASE COLLECTIONS

The concept and importance of active and base collections have been amply stated and given strong emphasis by both the National Plant Germplasm System (NPGS) and the International Board for Plant Genetic Resources (IBPGR). An active collection contains plant accessions that are available for distribution, evaluation, and documentation. Thus, accessions must be regenerated and multiplied to maintain diversity in the collection and to supply plant materials to users (14). An active collection requires methods of storage that retain the viability of samples for short to moderate periods of time (i.e., from a few weeks to several years). For active collections, clonal lines are usually maintained as field collections in either orchards or plantations or in screenhouses or greenhouses. The use of in vitro methods for maintaining plants has been advocated as an alternative route or as a safety backup for field materials. Distribution of materials from active collections is in the form of seed, budwood, cuttings, divisions, in vitro plants, and, perhaps, even as collected pollen.

A base collection, by contrast, is intended for long-term conservation of diversity (gene pool) and materials are not intended for extensive distribution. It serves, rather, as a duplicate store of diversity for a given accession should the active collection be depleted (14). Preservation of base collections requires methods that retain viability for long periods of time to minimize loss of diversity during the regeneration phase and reduce costs of regeneration and frequent viability testing.

PRESERVATION

Historically, many crops have been maintained clonally for reasons related to cultural aspects or the failure to form viable seeds. In some instances, clones are more productive than seed-derived lines, especially where standardization and uniformity are desired. Reasons for clonal maintenance of lines from the standpoint of germplasm preservation usually involve considerations of one or more of the following (16): a) lines do not form viable seeds (e.g., garlic); b) seeds produced are recalcitrant—that is, they have short storage lifetimes and do not tolerate much drying (13); c) seed sizes are so large that storage of sufficient numbers would require large amounts of space (e.g., coconut); d) lines possess long juvenile periods, hence flowering and seed formation are delayed (woody plants); e) clones are normally heterozygous (and the species heterogeneous), hence continued crossing will break up unique genetic combinations that have evolved (16).

Methods have been developed over the years so that clonal materials in active collections are in an available form for distribution and use by breeders. Different approaches are desirable for efficient and effective preservation of active vs. base collections. Strategies for maintaining clonal base collections are not as well-developed compared to those for seed. Some consensus, however, is emerging. Apical or axillary buds from in vivo or in vitro plants could be stored at cryogenic temperatures to maintain the clone (28, 35, 47, 63). However, long-term storage also could be accomplished through production of a seed population if plants are fertile and have no major incompatibility problems and if generated seeds are orthodox (33, 53). Orthodox seed are easy to handle, and viability can be maintained for many years through control of seed moisture and storage temperature. The genetics of many clonal crops are becoming known, but, compared to major agronomic crops, population structures (wild/domesticated) are still poorly defined, which complicates collection and maintenance strategies. Whether seed or clonal preservation would be most desirable has to be examined on a crop-by-crop basis. Many considerations must be assessed. For some crops, crossing to produce a seed population may lead to loss of genetic information if lethal factors are exposed. Herbaceous clonal lines are usually easier to maintain as seed populations than are woody lines. Overcoming graft incompatibilities and long juvenile periods may facilitate using seed materials from woody lines for some breeding projects. Numerous other examples could be given. The acceptance of seeds for base collection preservation of a given crop may reduce efforts into development of other methods for long-term preservation (i.e., growth-restrictive conditions for in vitro plants).

CLONAL PRESERVATION

There are many genetic issues related to collection and maintenance of species that we held as clones, but this discussion will stress maintenance. Collection is, in principle, the same among different crops, assuming something is known of the species' breeding system and population structure. There are, however, many practical considerations that differ in obtaining adequate sampling among individuals and populations. Actual collections of diversity for many crops, particularly those of minor importance or whose members lie within areas not easily explored, probably are not an adequate sample of existing diversity (9, 29). Adequacy of collection differs considerably among species.

Once materials have been collected, the genetic composition must be maintained by the best preservation and propagation procedures available. This statement is obvious, but the balance between practical and optimal often is based on costs and time available.

Accurate characterization of the clone is required to answer questions related to genetic stability. Characterization and evaluation would include data on morphology and phenology, agronomic or horticultural performance, physiological aspects, and electrophoretic analyses. The latter might be the most useful for germplasm purposes (40, 51, 55). For example, nonspecific protein and isozyme analyses have been used to identify and distinguish cultivars within many species (55). Methods are also being developed for electrophoretic and probe analyses of restriction enzyme fragments

(26, 44). Analyses of both nuclear and organellar genomes would be useful in characterizing lines; but, until such detailed, composite characterizations are available, it is difficult to know if an accurate job of maintaining clonal integrity is being done. This information also is necessary to determine frequencies of mutation (or other types of change) that might occur.

What type of change can one expect to see in a clone over time? This question, although simple in concept, is not answered easily if one is concerned with the exact nature and with the rate or frequency of change. The variation that is observed in a clone usually is due either to mutation, rearrangement of a chimera, or epigenetic change (37). In addition, changes in disease status of a clone (either becoming disease-free or disease-infected) can produce individuals with an altered appearance. Expression of mutations depends on ploidy and the nature of the mutation. Some may only be expressed in the progeny; others may express themselves in the affected individual, but only after several budbreak generations. Estimates of frequency depend on the ability to discern change. A number of quantitative traits may also be altered, but go undetected (taste, growth rates, etc.). Determination of the type of variant one has requires detailed genetic analysis and knowledge of the pattern of inheritance. Frequencies of occurrence in populations or within clones are usually not determined, and, hence, it is difficult to compare frequencies and types of change observed in field collections with those that might occur in in vitro collections.

The uncertainty of whether change has occurred also is manifest in preservation strategies. Since any single individual (or isolated propagule) may be a variant, isolates from different representatives should be made if a desired clone is to be preserved. This represents the most cautious, conservative approach to maintaining the clone. Practical issues restrict the number of individuals that can be maintained within a collection.

Clonal germplasm preservation usually is accomplished ex situ, although preservation in situ is considered for populations of related wild species.

In situ maintenance

This process involves the use of natural sites for preservation of materials (22, 30). An argument for this route is the continued evolution within a species of particular traits, including resistance/tolerance to disease or stress conditions, providing sufficient land and population sizes are used. There are many problems in establishing this type of preserve, for example, cost, political issues, and size and maintenance aspects. In particular, there is need to define specific conservation objectives. One must know the diversity of materials being conserved for application and effective management. This maintenance is best considered for wild relatives of many crop and forest species, especially in subtropical and tropical areas. Loss of germplasm in these areas is occurring rapidly, and international cooperation is needed for effective management.

Ex situ maintenance

This is the usual system for clonal maintenance and encompasses field gene banks such as orchards, plantations, and greenhouse and screenhouse collections. Field gene banks assure that a ready supply of usable materials in a mature state is available for distribution, characterization, and evaluation. However, field gene banks have the potential risk of germplasm being lost due to a disaster, and large amounts of space and labor are required to maintain only a small proportion of the diversity. It is doubtful that an adequate support would be available for the effort necessary to maintain the integrity of clones and supply distribution materials if collections were much more extensive. There is a low level of duplication of clones at a location, often only two or three individuals, and minimum duplication among locations. Disease or stress could easily eliminate all individuals of the clone. Both field and greenhouse plantings are maintained for many lines to minimize possible loss and disease spread. Little information is available on the stability of lines or frequency of mutants occurring under field gene bank conditions.

Preservation of in vitro plants and the cryopreservation of axillary buds from in vivo or in vitro plants are two areas of interest for base collections.

In vitro plant preservation

In vitro techniques are advocated for germplasm preservation because of the extensive development of plant cell, tissue, and organ cultures, and their application to many species (1, 20, 50). Some clarification and cautious use of terms are necessary to state what materials should be preserved in this fashion and the exact manner of preservation.

The term in vitro culture can refer to any proliferation maintained in sterile culture. There are ample data in the literature for many species that plant regenerants derived from adventitious buds or somatic embryos formed directly on leaf, stem, root, or other explants, or formed on callus, may exhibit considerable somaclonal variation (6, 15, 18, 19, 39, 43, 49). The frequency of variants differs among lines and species and with the extent and number of passages of callus. This potential for variation poses problems for maintaining exact clonal identity through the use of undifferentiated tissues. Thus, germplasm preservation in vitro must be accomplished by use of plants that initially were derived from meristem tips, shoot tips, or buds isolated from field or greenhouse materials and in which the tip developed directly into the plant. These in vitro plants (hopefully disease-free or at least tested) then are stored in vessels and subsequently micropropagated.

The micropropagation procedure should give clear evidence that multiplication occurs from existing axillary buds and not from adventitious buds. Methods for multiplication and preservation must be applicable to a range of genotypes and not impose any selection pressures. Unfortunately, conclusive evidence for most of the aforementioned points is lacking for most systems, and this is especially true for woody materials.

In vitro plants obtained and maintained by apical or axillary bud propagation are useful for either active and base collections, or both (31, 32). Morel (42) recognized early that in vitro plants are valuable for germplasm purposes by virtue of the control of their growth, ease of multiplication, and ease of retaining the disease-free state. The advantages of in vitro plant culture for germplasm maintenance include small amounts of space for storage, possibility of freeing plants from viral or other diseases, increased rates of multiplication, micropropagation of species often difficult to propagate, availability of propagules throughout the year, storage of plantlets for extended periods, and ease of shipment. These points have been described in review articles (16, 32, 49, 62).

Even with current understanding and level of technological development, use of in vitro plants for preservation is not without problems. These problems are usually not elaborated, but need to be considered in developing germplasm preservation procedures. They include susceptibility to disaster, human error, intensive labor requirements for in vitro collections properly maintained and monitored, difficulties in establishing and maintaining cultures, genotypic specificity at all stages of the in vitro process, use of growth-restrictive conditions, and the possibility of producing aberrant plants.

The first two points bear on the safety of maintaining in vitro materials. Storage of all materials within small growth chambers or something similar may be just as susceptible to problems as storage under field conditions. For example, mite infestation or equipment failure can cause the loss of cultures. Human error in mislabeling, media preparation, or misidentification of in vitro plantlets also can occur over time, since there are few morphological characteristics to give a simple check as to reliability. Identification of materials by growing out plants would be time-consuming, especially for species with long generation times. Thus, extensive electrophoretic characterization of the in vitro plants and periodic testing are necessary for reliable maintenance. Labor involved in these processes might be considerable for a large collection if all the procedures are carried out in a critical fashion.

Establishment of an in vitro plant is often difficult, and considerations of stock plant condition, physiological stage, and maturity must be assessed. Genotypic specificity for ease of establishment often occurs at this stage, as well as for the propagation and rooting

stages. Woody materials often have distinctive maturity stages that can be manipulated for use in establishing cultures (24).

In vitro maintenance of certain lines may be difficult. Vitrification, the glassy appearance of some shoots maintained in culture, is usually a physiological disorder (38). These plants often do not grow, can not be acclimated, and are useless for germplasm efforts. Some genotypes seem predisposed to vitrification, and the condition is not always rectified by manipulating the culture environment.

Acclimatization of some materials is also difficult, and some losses could be expected to occur during transplanting (45). The role of an active collection is to supply materials in a usable form, hence, the inability to acclimatize also limits access to the germplasm. Eventually, these culturing problems may be solved successfully, but such studies require time to deal with diversity of materials.

Growth-restrictive temperatures or media formulations are proposed to lengthen the time between transfers and give enhanced storage durations, thus reducing labor and introduction of errors (32, 62). These conditions have been used successfully to date on easy-to-propagate materials such as strawberry and potato, and their eventual application to diverse lines appears feasible. Again, genotypic specificity may be a problem. A crucial question is whether growth under these conditions might be a selective process, allowing loss of some lines, rearrangement of chimeras, or possible expression of abnormal types. There are few data on this point for in vitro plant cultures stored under growth-restrictive conditions, but limited experience with strawberry and potato has shown no major problems.

Most reports for meristem culture and micropropagation show production of true-to-type plants (e.g., ref. 46). But a few reports suggest that some lines produce an increased frequency of abnormal plants (34). In some instances, this increase may be due to environmental factors, and the altered appearance will be transient (17, 59). In other instances, altered field appearance may be due to increased vigor or proliferative characteristics of in vitro plants (54). Increased use of micropropagation in the floriculture and nursery industries attests to a high frequency of faithful reproduction if careful attention is paid to critical steps in the procedure. Occasional problems are noted; for example, chimeral rearrangements might occur during meristem culture (7). Some instances of extreme variability have been observed in field plantings from in vitro plants, but such information is not published, and studies appear not to have been made to explain such variability.

Cryogenic preservation

Ex situ preservation can also be accomplished by storage of buds, shoot tips, twigs, and cuttings at low temperatures, where diffusion and chemical reactions are so slow that little loss of viability is expected to occur over time (41). Liquid N (LN) temperature (−196°C) or that in the vapor phase above LN (about −150°) often are used because it is a suitable, relatively cheap cryogen. It should be emphasized that, in dealing with hydrated tissues, as contrasted to seeds, losses in viability usually do occur in taking samples to and from these low temperatures.

The advantage of cryogenic preservation is that propagules can be stored indefinitely without the need for subculturing. Therefore, the costly processes of plant regeneration and frequent viability testing are minimized.

There have been considerable advances in the study of the cryobiology of plant materials over the past 10 to 15 years (52). There are still questions about what freezing injury is and how it is alleviated by the use of cryoprotectants (21), but empirical methods have been applied to many different plant materials, including protoplasts, cell suspensions, callus, tissue and organ fragments, and buds and shoot tips, with varying levels of success (35, 47, 48, 63). The following comments are restricted to bud and shoot-tip systems, since these are the desired propagules for germplasm preservation strategies.

Buds and shoot-tips have been frozen and plants retrieved from a range of materials, including asparagus, brussels sprouts, carnation cassava, chickpea, pea, peanut, potato, apple, currants, gooseberry, pear, raspberry, strawberry, and tomato.

A number of issues must be addressed before this technology can actually be used for germplasm preservation. There is often an unsuitable percentage of survival for buds or meristem tips after low-temperature treatment. This percentage is often 60% or lower, although considerable variation occurs among reports. What constitutes a minimum, usable percentage of survival for a base collection? Although there are no guidelines, more than about 80% seems desirable. This level would reduce the number of buds required per sample and number of samples frozen per accession.

There is considerable experimental variation in survival. This variation suggests that all of the factors involved in a cryogenic protocol have not been optimized (35, 36, 47, 63). Further examination is necessary to produce a method that is dependable and useful for a wide range of materials.

The pattern of growth of treated buds varies considerably among species. This variation ranges from direct development of the treated shoot-tip or bud into a plant [e.g., apple and some other hardy materials (48)], to development of adventitious buds or somatic embryos directly on the treated shoot-tip or bud [pea (25), carrot (61), *Solanum etuberosum* Lindl. (56)], to development of a small callus prior to adventitious bud initiation [potato cultivars (57)]. Potato is probably the best-studied system, and it is quite apparent that growth-media formulations affect subsequent growth response (27, 28, 57). For nonacclimated materials, there is often a differential survival of cells within the low-temperature-treated bud, and hence callus, somatic embryo proliferation, or adventitious bud formation may occur before plant regeneration. A callus phase prior to shoot initiation is undesirable, since callus potentially increases the frequency of variants. The manner of development of low-temperature-treated buds needs to be described for other species, especially woody ones. In addition, chimeras will be impossible to preserve unless the whole bud survives and develops directly into a plant.

There are few analyses of regenerants obtained from buds or shoot-tips stored under liquid N conditions. Such information is essential to determine if cryogenic and culturing protocols are effective in maintaining clonal integrity. Once in vitro plant cultures are established from a thawed propagule, the same considerations previously discussed for in vitro plant maintenance must be evaluated. Analyzing variation from regenerants formed from buds that were originally isolated from in vivo plants, but required in vitro culturing after freezing, may be more difficult than analyzing variation from buds derived from in vitro plants.

There is a lack of information to demonstrate applicability of a cryogenic protocol for the many genotypes that would be in a collection. It is reasonable to expect genotypic specificity in response of treated buds to cryogenic and culturing regimes. In tuber-bearing *Solanums,* bud survival was usually high for many cultivars and some species; however, the capability to regenerate plants on a given medium was restricted (57). There is also the possibility that certain cryoprotectant combinations may be toxic to selected genotypes.

Strategies for cryopreservation must deal with materials that have varying degrees of hardiness and capabilities to acclimate. Plants often are formed directly from buds obtained from in vivo plants in an acclimated state (e.g., apple). Acclimation of in vitro plants needs further study to see if this is a feasible route for increasing cell survival within the bud (12).

Although there is concern about possible mutagenic effects from the cryogenic protocol, few reports directly deal with this issue for higher organisms. However, in bacteria, freezing and thawing in the presence of a cryoprotectant and extended storage did not increase the frequency of revertants in the Ames system (4). In mammalian cell lines, the frequency of sister chromatid exchanges was not increased (4). There was no loss of plasmids from *Escherichia coli* with storage in liquid N (8). Use of low-temperature-stored semen has not led to reports of demonstrable increases in abnormal individuals.

Additional research is necessary, however, because of insufficient studies. Also, observations that freeze-drying is mutagenic to bacteria (3), that dimethyl sulfoxide (DMSO, a cryoprotectant com-

monly used for many organisms, including plants) has gene activation effects in mammalian systems (4), and that DNA in situ may be damaged if no cryoprotectants are used (10, 11, 60) provide cause for caution. Information on the effects of DMSO on plants is sparse, but it is known to interfere with metabolism (5) and cytokinesis (58).

Normal DNA repair processes are not functional in materials stored at low temperatures; hence, the question exists as to what periods of storage are possible at low temperatures before background radiation causes genetic damage. The typical approach has been to use high levels of radiation for short periods of time and then extrapolate to years of possible storage with background levels of radiation. The few studies in this area are not with plant systems, but suggest that several centuries of storage are possible before effects are noted (2, 23).

The previous discussion is to highlight questions that require answers before cryopreservation is used on a large scale. Indeed, with recent advances in understanding freezing injury and the role of cryoprotectants, the expectation is that techniques can be adequately developed for preservation of base collections.

SUMMARY

Some of the points for active and base collection maintenance of clonal materials have been listed. There is a lack of data on genetic changes that occur during preservation and, as such, it is obvious that more information is needed if systems are to be constructed that maintain genetic diversity and clonal identity. This information will have to be developed on a crop-by-crop basis. At present, in vitro cultures are not used as the sole mode of maintenance for clonal systems. In vitro systems are used as backup collections, but without clear guidelines of what procedures are necessary and what levels of replication and duplication are needed as adequate safeguards. These considerations, along with guidance on what diversity of materials are to be stored, must be answered by the user community. For the present and near future, clonal collections should use both field and in vitro maintenance, and at least one duplicate of the collection should be held.

Theoretical and practical problems associated with the use of cryogenics for storage of hydrated propagules require further study before application to many clonal lines is possible. Cryopreservation of buds from materials that have the physiological capacity to cold-acclimate should be feasible in the near future if there is evidence of stability in the regenerants. Guidelines for implementing a cryopreservation system for germplasm base collections, however, are needed. These would include handling, storage systems, regrowth and culturing information, replicates needed, duplication of collection, and testing and genotype verification.

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