Germplasm centres and issues of seed conservation

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Abstract

The strategic value of seeds and seed genebanks is reviewed in a New Zealand context. This country, more than most, must recognise the values of two sources of plant biodiversity – native and introduced. Seed storage is a way of conserving large amounts of genetic diversity in a small space. The limiting factor to sustainable seed genebank operation is the capability to replace the seed samples of populations before the seed dies. This is a function of seed storage life and the number of seed lines that can be replenished annually. Scientific aspects of seed storage and seed replenishment are briefly reviewed. The role of core collections and the sampling research required to achieve them, especially using molecular markers, is summarised. It is concluded that, given the importance of introduced species plant genetic resources to New Zealand, research into the most efficient conservation strategies for seed genebanks should continue and, if possible, expand.

Additional key words: *biodiversity, core collection, molecular markers, seed replenishment, seed storage*

Introduction

This paper is about the strategic value of seeds, and seed genebanks, especially to New Zealand. Briefly, the strategic value of seeds to New Zealand lies not only in the seed industry and the use of seeds in agriculture, but also in the strategic importance of biological diversity or genetic diversity to this country and the role of seeds in the conservation of biodiversity.

The Strategic Value of Plant Biodiversity to New Zealand

New Zealand has an indigenous flora that has enormous value based largely on its uniqueness and indirect environmental economic importance, but it is of no value whatsoever for feeding, clothing or housing of a large human population. New Zealand has an equally large introduced flora that, by contrast, provides the basis not only for feeding, clothing and housing New Zealanders, but also is the basis for the national prosperity through agricultural and forestry export industries.

New Zealand, more than most countries, must therefore recognise the values of two sources of plant biodiversity – the native and the introduced. The indigenous flora and associated ecosystems must (at all costs) be conserved for their uniqueness and indirect economic value. The introduced flora, on the other hand, must be conserved for its direct economic value.

The Value of Introduced Biodiversity to New Zealand

Almost the entire agricultural, horticultural and forestry industries, which account for a large proportion of New Zealand's domestic economy and its foreign exchange earnings, are dependent on introduced plant and animal species. There is no realistic vision that New Zealand will have an economy based on anything other than biologically-centred industry. Thus, the biodiversity of other countries is of primary strategic importance to New Zealand.

There are two primary reasons why New Zealand should conserve introduced genetic diversity and continue to import more - for insurance, and for continued competitive growth.

New Zealand must maintain its production base from year to year against the threat of new pests and environmental changes. This requires new plant varieties derived from new genetic diversity. Thus, genetic diversity is required just to sustain current production levels.

Equally importantly, any initiatives to develop new biological industries require new species or new varieties of old species. Thus, genetic diversity is also required for competitive growth of the biological economy.

The Value of Seeds in Conservation of Biodiversity

Plant genetic diversity can be conserved by retaining dynamic ecosystems and protecting the genetic variation in living populations. This is referred to as *in situ* conservation, and this is the major focus of New Zealand's strategy to conserve its indigenous biodiversity.

Genetic diversity can also be conserved *ex situ*, i.e., by growing plants in nurseries outside of their normal environment or by collecting and storing seeds. *Ex situ* conservation is the major focus of New Zealand's strategy to conserve its introduced biodiversity.

Seed storage can conserve huge amounts of genetic diversity in a small space. As gene stores, seeds are remarkable. Seed storage is also a way of storing biodiversity in a manner that minimises threat to the native ecosystems.

The Margot Forde Forage Germplasm Centre, located at Fitzherbert West, Palmerston North, is one of four major (and many smaller) facilities in New Zealand designated to conserve biodiversity as seeds. The emphasis of the Centre is grassland plants. Other centres exist for food crops, horticultural crops and forest species.

Conservation of genetic diversity using seeds requires consideration of the following aspects:

- The replenishment bottleneck
- Strategies for replenishment of more lines
- Optimising seed storage life
- Optimising replenishment numbers
- Physical containment
- Population sampling
- Collection size - genetic diversity and core collections

The Replenishment Bottleneck

The limiting factor to sustainable genebank operation is the capability to replace the seed samples of populations before the seed dies. To achieve this replenishment most genebanks grow each population and harvest fresh seed, at intervals depending on the life-span of the seeds. The sustainable size of a collection is the product of the life of the seed and the number of populations that can be replenished annually. Seed life depends on several factors including seed quality, and storage conditions. For most genebanks, storage conditions for working samples involve higher temperatures (0-4 °C) than those for long-term samples (-20°C or lower) and consequently these seeds have a shorter storage life. The logistics therefore change depending on whether the role of the genebank is to store material, or to both store and distribute seeds for breeding or research purposes. Most genebanks have storage conditions that will keep seeds alive for 30-50 years and are able to grow 100-500 populations annually for fresh seed. Thus, on average, the sustainable genebank size varies from 3,000 to 25,000 accessions.

Most genebanks are responsible for conserving populations of several (often many) species. Even relatively specialised genebanks (e.g., cereals) are required to conserve samples of a minimum of 25 species when wild relatives are included while less specialised facilities like the Margot Forde Centre are required to store up to 2,000 species. Thus, especially in the latter case, a limit of 3,000 entries would greatly restrict the amount of diversity of each species that could be maintained.

Strategies for the Replenishment of More Lines

It is therefore very important to optimise seed life and replenishment numbers so that numbers closer to 25,000 can be sustainably conserved.

Optimising seed storage life

Longevity of seed in storage depends on provision of an environment in which physiological and pathological deterioration is minimised. Storage affects seed vigour more than viability (Wilson, 1994). Environmental factors which can be controlled include temperature and moisture.

Temperature

Longevity of seed is negatively related to temperature, presumably because the rate of chemical reactions approximately doubles as temperature increases by 10°C. For high temperatures this relationship is exponential but there is uncertainty about the relationship at temperatures below ambient (Wilson, 1994). Genebanks usually use temperatures of 0-4°C for medium term storage and -20°C for long-term storage. Cryogenic storage (liquid nitrogen, -196°C) may provide longer storage and is estimated to cost only a quarter of conventional deep-freeze storage over a 100 year period (Stanwood and Bass, 1981). For crops with recalcitrant seeds or no seeds, cryopreservation of tissues or "artificial seeds" is giving some success (Tessereau *et al.*, 1994; Withers, 1993).

Humidity

Seed moisture content is the most important determinant of longevity in storage (Wilson, 1994). A rule of thumb (Harrington, 1960) is that storage life doubles for each 1 % decrease in seed moisture content. However, there appears to be a lower limit beyond which this does not apply (Ellis *et al.*, 1989; Vertucci and Roos, 1990), and drying seed below this limit may be counter-productive. Methods for determining moisture content of stored seeds are given by Ellis *et al.* (1985) and Wilson (1994).

Genebanks must control relative humidity (RH) as low storage temperatures cause RH to rise. At 25 % RH and 4°C seeds equilibrate to a moisture content that is optimal for long-term storage regardless of the species (Vertucci *et al.*, 1994; Clark *et al.*, 1997). Where low RH is not provided, dried seed is usually sealed in aluminium-polyethylene.

Storage of grass seeds requires survival not only of the seed embryo but also of any accompanying endophytes. Endophyte viability is more sensitive than seed germination to effects of high temperature and seed moisture (Rolston *et al.*, 1994).

Other factors

Attack by free radicals is one possible cause of seed deterioration in storage (Wilson, 1994). Studies of the effect of antioxidants to counter this have been equivocal (Wilson, 1994).

Optimising replenishment numbers

The low number of seed lines that can be replenished annually is a major problem confronting genebank managers as it limits the numbers of accessions that can be sustainably held. Alternatives to regeneration *ex situ* include repatriation of germplasm and re-collection from the source population *in situ*, and conversion of samples to frozen DNA libraries. However, for various reasons, including threats to the integrity and difficulty of access of original populations, and the failure of DNA libraries to capture information at a genome level, *ex situ* regeneration must be maintained.

The problem can be divided into two elements. First there is the physical resource needed to achieve reproductive isolation of hundreds of populations that are cross-pollinated by wind or insects. Second, these populations must be large enough to satisfy population sampling requirements that Brown *et al.* (1997) have described as "the geometric tyranny of recurrent regeneration". The containment facilities must be of a significant size and are, therefore, expensive. There is also the question of the effect of the replenishment environment on population shift.

Physical containment

This is largely a financial cost factor –purchase and maintenance of containment facilities and employment of technicians to operate them. Generally the more expensive methods (hand-pollination, isolation in pollen or insect-proof tents) are the most effective for maintaining effective population size and the genetic integrity of populations, while less expensive methods sacrifice genetic integrity (Clark *et al.*, 1997). With cross-pollination in the field it is not possible to grow large numbers of accessions of the same species without some contamination.

Collections of 'wild' species, such as forage and medicinal plants, are generally more costly to regenerate than those of cultivated species because of their life history, breeding system, genetic structure, ecology and lack of domestication (Brown *et al.*, 1997).

Population sampling

The key to successful accession maintenance is the degree to which initial genetic diversity is retained after regeneration. The theoretical and scientific basis for optimising germplasm regeneration was reviewed by Breese (1989), and has been updated for wild germplasm by Brown *et al.* (1997). Wild species tend to be polymorphic and effective population size must be large to minimise random allele loss by drift. Biases in parental contribution to the next generation tend to reduce effective population size. Such biases are prevalent in natural populations and so plant numbers much higher than estimated numbers may be needed to minimise allele loss.

The probability of maintenance of allelic diversity is a function of the variance effective population size. For wild, outcrossing species, this is maximised by expensive controlled pollination methods (Yonezawa *et al.*, 1996) that are often not practical on a large scale. Brown *et al.* (1997) have shown that each subsequent cycle of regeneration requires a three-fold increase in the number of plants harvested if all of the alleles of interest in the original population are to be retained with 95 % certainty. Thus, for alleles present initially at a frequency of 0.05, successive regeneration cycles require 30, 90, 270 etc. plants. Even for relatively common alleles, the aim of keeping them all is unrealistic.

Genetic variation in the wild is often clinal over long geographic distances (imposed, for example, by climatic trends), but there are also sharp environmental discontinuities that lead to discrete adaptations (Breese and Tyler, 1986; Allard, 1988). In self-pollinated plants, adaptation to new environments occurs by an accumulation of large synergistic complexes of favourably inter-

acting alleles at many different loci (Allard, 1988). In cross-pollinators or wild species, the process is similar but more diffuse because outcrossing tends to break up the combinations at each generation. Closely linked associations are nevertheless identifiable, and conservation of these favourable associations is clearly of priority in species where discrete adaptational differences are important.

For practical purposes, Brown *et al.* (1997) recommended that plant numbers should be at least double the number of parents of the previous replenishment generation. Alleles will be lost but, as long as contamination is prevented and purity is maintained, this is the best that can realistically be achieved. Then priorities should be decided on the basis of maintenance and maximisation of genetic diversity between and within populations.

Collection Size – Genetic Diversity and Core Collections

No matter how large the genebank budget is, it is inevitable that there will be a need to prioritise entries and to select those that will be retained and those that will not be replaced. One approach to this is retention of a relatively small proportion of the entries (a working or core collection) that represent most of the important genetic diversity of a species.

In practice this concept is not simple and methods for achieving it are currently being developed from sampling theory, aided by molecular marker analysis to enable the genetic consequences of various strategies to be compared. These methods have recently been reviewed (Hodgkin *et al.*, 1995). Not surprisingly, because adaptational forces are strongly geographic in nature, geographic origin is a major factor in sampling core subsets and stratification by log-frequency of location of origin ensures that there are adequate samples from the less common locations (Spagnoletti Zeuli and Qualset, 1993). Multivariate analyses of phenotype can provide further stratification, but the value of this is doubtful even if highly heritable characters are used.

Wild species offer a particular set of challenges for core collection sampling because of unequal diversity and differentiation among accessions. Molecular markers can be valuable tools to identify allelically rich accessions to aid sampling (Schoen and Brown, 1995). As an example, multivariate analyses of a diverse set of white clover (*Trifolium repens*) varieties showed that plant phenotypes fall into about nine groups (Caradus *et al.*, 1989). Each group contains a large range of geographic origins and hence clear differences in adaptation and genetic variation. Griffiths and Williams (1999) adopted the molecular marker approach to identify genetic diversity within and between white clover populations from multivariate groupings of Caradus *et al.* (1989). They have run AFLPs on populations sampled to cover the range of morphological variance. Two of the populations were phenotypically similar ladino clovers. Both had a common geographic origin in northern Italy but one population had been taken to the USA in the 1890s and since then had been cultivated as Californian Ladino. The other stayed in Italy as Italian Ladino. The two populations had retained their phenotypic similarity. A third population from Kent (UK) was quite different from the ladinos, having smaller leaves, higher cyanogenesis and no known ancestral relationship.

It was predicted that the two ladinos would be genetically similar and that the Kent population would be genetically different. In sampling of a core collection, it was therefore considered that one of the ladino populations would be redundant. When this hypothesis was tested using AFLP results showed, as expected, that the Kent population was different from both the ladinos (genetic similarities were 0.61 and 0.56 for Kent-Italy and Kent-USA, respectively). However, when the ladino populations were compared, they were as different from each other as they were from Kent (genetic similarity 0.61). One hundred years geographic separation appeared to have resulted in wide genetic divergence of these populations. This was surprising, given their fairly recent common geographic origin and phenotypic similarity. It was apparent that phenotypic assessment was of limited value in genetic diversity measurement while molecular markers could be a very valuable tool. These issues are discussed further elsewhere (Griffiths and Williams, 1999).

It is clear that more research on seeds as stores of biological diversity is required so that genebank management procedures achieve the maximum retention of genetic diversity. This applies especially to wild species, as opposed to domesticated crops, which have received most of the effort to date. For New Zealand, which is so dependent on the conservation of germplasm of introduced species, there is a need to expand research in this area so that only the most efficient methods are used for conservation of genetic resources required for the future.

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