Indian J. Plant Genet. Resour. 29(3): 268-271 (2016)
DOI 10.5958/0976-1926.2016.00043.7

Gene Banks: Management of Genetic Erosion in *Ex Situ* Collections

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Introduction

Plant genetic resources are the fundamental source for crop improvement which is being conserved *in situ* and *ex situ*. While *ex situ* is static form of conservation of plant resources, *in situ* is dynamic; representing diverse populations (including both alleles and genotypes) and aids the evolutionary processes of gene flow between different populations and natural and artificial selection/ interaction in heterogeneous environments. Apropos to conservation of biological diversity *ex situ* in post CBD scenario, but with special reference to genetic erosion there in, also gained immense significance in the wake of IP regimes among all the CBD member countries. There exists imminent danger to the very existence of rich diversity in nature in the wake of raising global temperatures and climate change. In the backdrop of several species becoming rare and endangered in their natural habitats, *ex situ* conservation of large chunk of diversity is though safeguarded to certain extent; the threat of erosion still haunts us as the genetic erosion in *ex situ* collections is looming large.

Historic Plant Explorers/Collectors

For many thousands of years, plants have travelled around the world along with people journeying to different countries for e.g. tomato, maize, pepper (chilli) (South America), banana (South East Asia), carrots (Afghanistan), potato (Andean region, South America), onion (Central Asia), wheat (Near East) and pumpkins (tropical America). Contributions of the following are worth mentioning.

- Queen Hatsheput (Egypt, 3500 years ago) – collected resin of myrrh plant and frankincense trees near Somalia.
- Christopher Columbus (Italy – in 1492) – Potatoes, sweet potatoes, maize, tomatoes, peanut, cassava, cacao, peppers, tobacco, beans and squashes.
- Thomas Jefferson (3rd President of USA) – Vanilla, tea, olives.
- Nicolai Vavilov (Russia) – Centres of origin.
- Carlos Ochoa (Peru) – Wild and endangered species of Potato.
- John George Jack (Canada) – Tree genetic resources.
- Jack Harlan (USA) – Centres of diversity.
- Gregory WC – Peanut germplasm
- Krapovickas A – Peanut germplasm
- Brown WL – Maize germplasm
- Hawkes JG (UK) – PGR science.
- Otto Frankel (Australia) – PGR science.
- Zeven AC (The Netherlands) and PM Zhukovsky (Russia) – Centres of origin.

In the Indian context, Emperor Akbar (1542-1605) established a mango orchard (*Lakhi Bagh*) in Darbhanga, Bihar during his regime. Emperor Ashoka (304-232 BC) patronized the establishment of fruit and shade trees in his kingdom.

Status of World PGR in Genebanks

Over 1750 gene banks and 2,500 botanical gardens conserve a total of 7.4 million germplasm accessions and 80,000 species respectively around the world. Currently, 7,74,601 samples are deposited at Svalbard, Norway by 53 genebanks. It is estimated that more than one third of the globally *distinct* accessions of 156 crop genera stored in genebanks as orthodox seeds are conserved in the global seed vault. Global holdings of commodity groups *viz*., cereals (31,57,578); food legumes (10,69,897); roots and tubers (2,04,408); vegetables (5,02,889); nuts, fruits and berries (4,23,401); Oil crops (181,752); Forages (6,51,024); sugar crops (63,474), fibre crops (1,69,969),

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medicinal, aromatic and spice crops (1,60,050); industrial and ornamental plants (1,52,325) and others (2,62,993) reported by FAO (2014).

Importance of Conservation

Ex situ conservation is designed to maintain genetic diversity available in and genetic integrity of the collected material, to avoid loss or degeneration. Use of conserved plant genetic resources have been successful in mitigating major challenges of abiotic stresses [e.g. chromosomal translocation from rye to wheat has conferred adaptation potential to wheat in marginal environments, Borner *et al*. (2000)], biotic stresses [e.g. rust resistance in wheat varieties by incorporation of single major gene that conferred resistance to specific races of rust pathogens (Borlaug, 1953)] and climatic changes [*e.g.* drought tolerant maize/stress resilient maize in Africa, (Chebotar *et al*., 2003)] and facilitated in increased crop production and productivity. Following appropriate protocols of regeneration of the *ex situ* conserved material is the critical step in maintaining genetic diversity and genetic integrity.

Ex situ collections are vulnerable to genetic erosion resulting in the loss of amount of genetic diversity and loss in genetic integrity, in terms of presence and absence of genes (alleles). Frankel (1975) reported in a survey of genetic resources that many collections had suffered genetic erosion due to hybridization, selection, genetic drift, unsuitable growing conditions, or human error during propagation. Quantification of genetic erosion requires time series analysis and using morphometric traits has always proven to be problematic (Thormann and Engels 2015). These changes were detected both by SNP and SNP haplotype analysis during regeneration in maize and significant differences in allelic frequencies were also reported in barley, rye, and *Brassica* (Parzies *et al*., 2000; Soengas *et al*., 2009), although in using limited number of accessions.

Challenges in *ex situ* Conservation

The conservation of the diverse plant genetic resources, as a possible source of wide gene/allelic diversity, under *ex situ* genebanks faces immense challenges and issues some of which are not entirely within managers' control. In *ex situ* crop wild relatives are threatened by changed habitat (soil, water and soil microbial populations etc.) agricultural/silvicultural practices, human selection etc., that impact on its fertility and seed production. The challenges are broadly defined by environmental factors for *in situ*/on-farm *conservation*. The extent of the impact of environmental factors varies from species to species; for example, whereas the cultivated plant species are faced with the challenges of habitat destruction, fragmentation, climate change and restoration efforts while the crop wild relatives of the plant species could be facing the challenge of climate change on a much bigger scale than the habitat destruction and fragmentation *in situ* (Guarino *et al*., 2011). These challenges precipitate into genetically less diverse populations of the plant species. Jump *et al*. (2009) stated similarly that, genetic erosion can check the resilience, evolutionary potential for adaptation in the short term and survival of any plant species in the long-term, in the face of rapid environmental change.

Reducing and managing the loss of genetic integrity and genetic variation of the conserved germplasm during regeneration is an important objective of genetic resource conservation programmes, i.e. reduction in genetic drift and genetic shift. Genetic integrity may be lost due to inadvertent selection and reduction in genetic variation due to cumulative bottleneck effects that would have started at the time collecting through small seed samples used for subsequent regeneration/ multiplication. The management of seed accessions in different genebanks can lead to differential loss of genetic integrity. Identification and rationalization of duplicate accessions in genebanks requires information on the genetic integrity of the accessions. In addition, different genebanks may use different methods of identification of duplicate samples and rationalization of collections which can lead to further genetic erosion. To recommend better practices for maintaining panmictic populations of germplasm accessions, studies on genetic integrity during seed multiplication and regeneration using molecular makers from other seed or clonally propagated crops can be useful.

Assessing Genetic Erosion in Genebanks

Factors Causing Genetic Erosion in Genebanks

Some of the factors causing erosion in *ex situ* collections are physiological changes in seeds, inappropriate storage conditions and management procedures, accidental errors/mixing of seed samples before regeneration, lack of adequate financial resources for maintaining collections, human behaviour (accidental destruction, fire), damage of field collection (animals, meteorological anomalies including natural disasters), armed conflicts, war, regeneration backlogs, economic instability, pest

and disease outbreaks, abiotic stresses (heat, drought), lack of resources and skills, and loss of samples during regeneration etc. Genetic erosion in gene bank collections depends on the quality and quantity of the original material stored, and on the conditions under which the germplasm is maintained, multiplied and regenerated.

Population Size at the Time of Collecting and at the Time of Regeneration

Number of accessions and their isolation during collection and at the time of regeneration are also indicators for genetic erosion. Small Populations are at risk of loss of alleles, increased inbreeding and extinction due to random environmental events. Secondly, sample size analyzed – small sample size may result in missing the allele detection, especially for rare alleles. Direct comparison of samples collected at different times in the *ex situ* collections are warranted to assess the genetic erosion.

Avoiding Genetic Drift

Genetic drift describes random fluctuations in the numbers of gene variants in a population. Genetic drift takes place when the occurrence of variant forms of a gene, called alleles, increases and decreases by chance over time. These variations in the presence of alleles are measured as changes in allele frequencies. Hence, the genetic purity of the conserved sample by avoiding genetic drift and inbreeding; the population size/population genetics theory of sampling at the time of regeneration to maintain the genetic integrity of the accessions in the genebank. Typically, genetic drift occurs in small populations, where infrequently occurring alleles face a greater chance of being lost.. Genetic drift can result in the loss of rare alleles and decrease the genepool. Genetic drift can cause a new population to be genetically distinct from its original population, which has led to the hypothesis that genetic drift plays a role in the evolution of new species.

Morphological and Molecular Characterisation

Stable and unique morphological traits should be effectively used for assessing the degree of genetic variation in the initial as well as regenerated *ex situ* conserved samples. Morphological characterisation of *ex situ* collections should be based on standardized format and the data follow internationally agreed descriptors list (Breese, 1989; Engels and Rao, 1995). Molecular characterisation of germplasm accessions is a useful tool for better management and to study genetic diversity and integrity of conserved germplasm. Previous studies using molecular tools have been performed on the genetic integrity of genebank accessions of some crop species during regeneration. The genetic integrity of the wheat accessions (Borner *et al*., 2000) and rye accessions (Chebotar *et al*., 2003) conserved in the gene bank were studied using microsatellite markers. Chebotar *et al*. (2003) found that there were 4 accessions had significantly different allele frequencies and nearly 50% of alleles identified in the original samples were lost in the regenerated samples. Also, interestingly, some alleles detected in the most recently propagated sub-populations were not observed in the investigated plants of the original seed stocks. Soengas *et al*. (2009) investigated the effect of regeneration on the genetic integrity of *Brassica oleracea* accessions based on simple sequence repeats (SSRs) and found that there were significant changes in the population structure and the allelic frequency at individual loci due to the action of genetic drift, directional selection, and possibly assortative mating. The new molecular marker system, known as single nucleotide polymorphism (SNP), is widely used in different crops *viz*., barley (Rostoks *et al*., 2006), maize (Yan *et al*., 2009) etc. Fingerprinting of genebank accessions can help manage genetic integrity of the germplasm accessions as well as the molecular diversity.

Recommendation

- Base collections, associated with information on traits of importance should be used as a reference point to estimate the extent of genetic erosion. Sample size at the time of collecting should be as large as possible.
- Sub sample (suficently large to avoid bottleneck effects) of the base collection to be used for evaluation and rejuvenation/multiplication.
- Define the genetic integrity at the time of collection and the method of maintenance (size of sample for regeneration, period of regeneration cycle, exposure to current stresses) for maintaining original genetic composition.
- Revisting the original locations of collection to assess the extent of genetic erosion.
- Research on protocols and scales and indicators (individual crop-wise) to maximize the genetic integrity.

- Follow proper regeneration protocols (Germplasm adaptation to environment, taxonomy, reproductive biology and genetic diversity studies are to be taken up).
- Modern molecular tools may be used to estimate the genetic erosion and the resultant diversity created during conservation should also be utilized for crop improvement.

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