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CONSERVATION OF PLANT GENETIC RESOURCES - SOME VIEWS

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Conservation is as vital as collection of genetic resources. How much and how best to conserve remain live problems. Conservation strategies have evaluation as one of the main components. Criteria to identify duplicates in collections still need to converge. There are chances of generation of new variability in 'static' collections of seed in long term storage. Collections are made continuously straining space availability in gene banks. Modern tools like molecular markers may add more to the number to be conserved. This is because collections whose phenotypic performance is very similar could be identified as distinct based on RFLP variation. This could cause a 'load' to the gene bank. A trade-off has then to be worked out among (a) novel genes to be conserved for their own sake (b) accessions with traits of potential use for breeding in the near future and (c) accessions with possible genetic differences as revealed by RFLP markers whose phenotypic expression is near identical in target environment. Further, in order to sustain the dynamic nature of conservation activity, serious thought should be given to conservation of germplasm complexes and populations. In this context, sampling strategies to conserve maximum with minimum number of samples also assume importance. This paper projects considered views and those arising from published work.

Conservation and effective utilization of plant genetic resources (PGR) are essential for global food security as was also stressed in SAREC Report (1992). A detailed action plan to conserve biodiversity and to prevent the extinction of any species including non-threatened stocks has been drawn up by IUCN/UNEP/WWF (1991). Special emphasis was laid on development of techniques to manage small populations of plant and animal species taking into account the need to prevent inbreeding and local extirpation resulting from accidents,

ecological catastrophes and climatic change. Today conservation strategy is based on perceived impending loss of biodiversity that includes interesting species, subspecies or even varieties. The focus is on loss of potentially useful resources such as plants with economic or medicinal value (Erwin, 1991). An area that causes concern and therefore deserves greater attention, is the degradation and conversion of the environments where the target taxa reside. In short, the horizon of the conservation needs is far wider than is being scanned. With scant knowledge on how much total diversity once existed, it is impossible to quantify the losses. But to save what is left of the world's priceless heritage of genetic diversity and to ensure that agriculture meets the needs of the next century and those to follow, we have no alternative but to conserve PGR (Anon., 1991). Yet, more than the need to conserve, how much and how best to conserve are live problems. There is no point in collecting material if it cannot be conserved, nor is there any point in conserving material which cannot be evaluated and utilised (Marshall and Brown, 1975).

COLLECTIONS IN GENE BANKS AND FURTHER NEEDS

It is estimated that size of germplasm holdings of major crop species available in various germplasm banks are: roots and tubers, 75,600; vegetables, 274,600 (Cruciferae 59,600; Cucurbitaceae 131,00); cereals, 11,96,214 (barley 280,300; maize 99,714; rice 212,200; sorghum 91,200; wheat 401,500); Grain legumes 185,140 (*Phaseolus* 70,750); forage grasses 84,200; forage legumes 127,900 (Holden, 1984). These estimates would, nonetheless, be higher now. As a resource-effective policy, IBPGR has been allowing collections only when it is a must. There are other facets of PGR conservation too; for instance (a) *in situ* conservation in natural or original habitat, (b) *ex situ* conservation in gene banks as seed, tissue or pollen, in field gene banks or in other live collections and (c) on-farm/community conservation (Anon., 1991). Viewed against this backdrop, large scale occurrence of duplicates in world gene banks is a severe constraint on purposeful conservation efforts. In some crops, the duplicate collection can probably be as high as 60 per cent (Holden, 1984). In some instances, duplicates of designated collections are encouraged, as a policy, to be maintained in more than one location as an insurance against disaster. But they form only a tiny proportion of the duplicate collections. Alternatively, the problem of identifying true duplicates when a large number of qualitative and quantitative traits characterize a collected genotype, is too complex. At the same time, increasing emphasis is also laid on conserving wild relatives of crops to sustain breeding efforts on incorporation of resistance to biotic and abiotic stresses. For example, the international rice germplasm centre holds over 2,300 populations of 25 wild species

including 5 species in 3 genera related to *Oryza* (Zuno *et al.*, 1988). Such activities stress, in turn, conservation of habitats of such wild species. Conservation of wild species, in its broad sense, has thus become an important activity of gene banks (Plucknett *et al.*, 1987). But outdated taxonomic classification, for example, of sorghum (Snowden, 1936) and poor adaptation of wild species to target environments as observed in *Oryza barthii* and *O. longistaminata* in Philippines and *Sorghum drumondii* in Illinois pose hurdles in their effective utilization (Harlan, 1984).

There is then a need for formulation of sampling procedures for species where exploration is a limiting factor, such as species threatened by extinction and those that occur in difficult and remote terrains. Some of them can be explored only once and the responsibility of the explorer is then immense. Further, conservation of too many accessions in one crop or species is necessary for maintaining the real variability, will curtail the resources and infrastructure for conserving another, probably more important, crop or species (Marshall and Brown, 1975). It has been conjectured that a barley collection of just 12,000 probably may contain a majority of barley genes (Creech and Reitz, 1971).

Conservation programmes need to be coordinated and systemaucally planned according to the crop specific limits and factors limiting the number of germplasm collections that can be made. Monitoring of resources for conservation will be facilitated if an estimate of the genetic variability trapped in the collections can be obtained with a reasonable reliability. It would simultaneously be of interest to take note of the conservation methods so as to minimise the decay of genetic variability. In particular, the progressive decay of variability over generations needs to be considered in relation to the strategy used such as selfing, planned crossing and the pollinating system of the crop.

GERMPLASM MAINTENANCE

Most of the PGR are maintained by growing them periodically in the field. In general, partial and complete outbreeders may exist in a germplasm bank. Depending on the seed requirement, a small sample (varying in size, may be 10 to 50 plants) is grown in the field. In the process, unless pollination is entirely controlled, new genetic variability can be generated. Further, where open pollinators are liable to show inbreeding depression (affecting survival), full sib mating is resorted to which will also result in slow erosion of heterozygosity. It is further recognised that distinctly different accessions grown in field, are subject to optimising natural selection against extreme variation that usually occurs despite developmental buffering (Travis, 1989). The argument that phenotypic plasticity would buffer the effects of natural selection

is not valid as it is now clearly known that genotypic performance in one environment can differ markedly from another. Such a response to varying environments can be considered to be adaptive only if it represents a mechanism for maintenance of relative fitness in the face of such variation. Then, in principle, evolutionary changes can result despite phenotypic plasticity (Thompson, 1991). Further, in the presence of some degree of pollen dispersal, particularly in populations with a low selfing rate, it has been shown by computer simulation that there is a chance for 'periphery effect' — the uneven distribution of genotypes at the periphery of a population compared to its central region (Yonezawa, 1989). This result adds to the problem of maintenance of populations. When extended, such concepts will hold to situations where (and, as usually, is) a number of distinctly different accessions are usually grown together in adjacent strips in field for maintenace. In particular, active gene flow between accessions can lead to differential loss of genetic distinctness.

GERMPLASM EVALUATION

Evaluation is a very important component of a gene bank's function and has a close relevance to conservation activity. The breeding worth of an accession can be realised only if it is evaluated including quantitative traits-morphological, physiological, biochemical and the like. They would serve as additional and essential data to passport information. Breeders from near gene bank location can straightway use the data for selection saving an evaluation at their experimental sites and those in areas far away from the gene banks can select accessions at the first stage to be evaluated in their environment for confirmation and second stage selection.

Multivariate analysis (for example, using D^2 statistic, principal components, factor analysis etc.), numerical methods (like dendrograms), modification of multiple range test of univariate means providing 'performance indices' (Arunachalam, 1992) and variations of the above methods are generally used for quantitative evaluation of germplasm accessions. The methods are well known and documented. By these methods, the genetic diversity among accessions is examined and closely related stocks are grouped into a cluster. If the constitution of clusters and the relative genetic distances of various clusters (formed out of a set of accessions) remain stable over environments (time and space), there is a scientific validity to maintain the accessions within a cluster as a germplasm complex. There would be divergent opinons regarding on how large an accession, in how many environments and over how many quantitative traits, the evaluation should be done. These questions would not admit of unique solutions. It may be observed however, that

such exercises are essential for every gene bank to restrict growth of duplicates and more importantly to make space to receive more, possibly very important, accessions.

MOLECULAR TECHNIQUES IN GERMPLASM EVALUATION AND CONSERVATION

The advent of *in-vitro* techniques particularly tissue culture has helped in widening the base of conservation and enhanced the scope for effective utilization of PGR. Conservation of germplasm as tissue cultures and their regeneration and evaluation have been well documented and will not be dealt with here.

Of recently growing interest, adding a new dimension to the scope of plant breeding, is the use of molecular markers in genetic identification. Such markers identified through restriction fragment length polymorphism (RFLP) have been suggested to have the potential of bringing more definiteness to conventional breeding. RFLP markers have been credited with unique advantages compared to conventional (morphological) phenotypic markers. RFLP-based linkage maps are projected to help identify molecular markers that would have close linkages with useful quantitative trait loci (QTL) of economic interest. The processes, concepts and methods have been frequently documented (Beckman and Sollar, 1986; Tanksley *et al.*, 1989). A series of papers on improving soluble solid content in tomato, the use of RFLP markers as a novel and viable technique of plant breeding and enormity of such molecular investigations on tomato has been described, (Paterson *et al.* 1991). RFLP markers have been used in germplasm characterisation by several workers. Five DNA probes used to evaluate 77 individuals from 5 lucerne germplasm groups revealed high polymorphism with 7-9 bands each. Cluster analysis suggested only three clusters among the 5 groups. Correspondence between conventional and molecular evaluation of diversity was made difficult by the small number of individuals and probes (Walton *et al.*, 1988). RFLP patterns suggested identity between modern cultivars and three wild accessions in soybean. The suggestion was that the wild accessions collected from China were of hybrid origin with *Glycine max* as the maternal parent in natural outcrossing (Close *et al.*, 1988). Extensive RFLP variation was found at the species, subspecies and varietal levels in *Brassica* (Song *et al.*, 1988). RFLP markers were also useful in genetic differentiation in cultivated rice (Takaiwa and Oono, 1991). Nevertheless how far RFLP markers can successfully substitute phenotypic markers, that indicate genotypic expression in target environment, is an open and debatable area. Papers projecting the other side of the RFLP advantages have also been appearing (Ellis, 1986; Blake *et al.*, 1991; Simmonds, 1991). The pros

and cons of employing RFLP markers in germplasm evaluation and conservation will only be discussed here. RFLP markers are DNA sequences detected by restriction enzymes. RFLPs are detected as bands in agarose gels and, therefore, do not have 'phenotypes' in the conventional sense. In general, they do not code for a gene function and cannot therefore have independent phenotypic expression. Most often their linkage to a continuously varying quantitative trait of interest is judged by LOD (a maximum likelihood) score. Such markers can be several, in principle, as a very large number of probe-enzyme combinations are available. Even though 'linkage' can be inferred between a marker and a quantitative trait in one stock, it cannot immediately imply that, whenever the presence of the marker is detected in any other stock, it would have the desired value of the quantitative trait. The reason may be that every quantitative trait is governed by a number of minor genes with small effects which are further modified by environment during expression. Further, most of the traits of breeding (and also gene bank) interest are quantitiave in nature. A few RFLP markers, which are codominant and nonepistatic, cannot predict the quantitative trait expression of an accession.

Moreover gene banks can suffer a possible 'load' by molecular marker-based germplasm diversity. Consider, for instance, two accessions, A and B which were evaluated for a number of quantitative traits and did not show significant differences in a target environment. They will be classified genuine duplicates and will be held in the germplasm bank as a single mixture, in general. If A and B were compared using say 20 probes, it is highly likely that they would be polymorphic for one or a few of them suggesting they are distinct accessions leading to individual maintenance. Extending this logic, it is possible that genuine duplicates would get individual identity by molecular markers causing the 'load' mentioned earlier. The argument that differences suggested by the RFLP markers can be genuine does not hold strength as phenotypic expression (which was earlier argued to be independent of the presence of markers) would be too uniform to uphold it. Further, the performance of an accession for more than one trait would be difficult to be judged by RFLP markers as then, we would be dealing with a multitude of them (taking into account that more than one marker may be linked to one quantitative trait). Moreover, the cost of using molecular markers for germplasm identification is far more at present compared to the cost of field-screening of germplasm accessions (Blake *et al.*, 1991). Thus with the present knowledge, there appears to be no safe premise to advocate the use of RFLP markers over conventional quantitative methods in germplasm evaluation.

SOME STRATEGIES FOR OPTIMISING CONSERVATION

Optimum conservation is a function of abundance of a target species, *a priori* knowledge of the frequency of alleles to be conserved, the number of sites where the target species occur, the distribution of rare alleles and the resource (time, cost, manpower) availability. Other than the last variable, it is most often difficult to get adequate information on others. Even when the status of all the variables is known beforehand, it is difficult to frame optimum strategies of sampling the target site. An ideal strategy is the one which helps to conserve maximum diversity with minimum number of samples. In addition, it is desirable to conserve at least one sample of each of the different alleles in the target species (Bennett, 1970; Marshall and Brown, 1975). This would demand information, for example, on whether the allele is highly or moderately frequent or rare. Some estimates place about two alleles per polymorphic locus occurring in intermediate frequencies in natural populations (Clegg and Allard, 1972) in *Avena barbata,* for example. However, such estimates are experiment-specific and cannot be generalised. Likewise, estimates on sample sizes necessary to represent a target species were attempted (Marshall and Brown, 1975). When no information was available on variation in a target species, a random sample of size 100 and when such information was available, a sample of size 50 plants per population were suggested. Those estimates contrasted the size of 200-300 plants suggested earlier (Bennett, 1970; Allard, 1970). Since none of those estimates were general enough (and dependent on the conditions of simulation of theoretical basis), it would seem that a safe strategy is to sample as many sites as possible using a realistic sample size constrained only by the available time and resources.

Conservation requires regeneration of PGR at periodic intervals to maintain seeds. With modern methods of seed storage including long term cryopreservation, the intervals for regeneration can be extended from a few to 20-25 years or more. In this process, loss and alteration of genetic variation through differential survival and selection during 'static' storage are factors to be reckoned with. Once the seed stocks are grown in field, the need to identify optimal mating systems and the size of progeny to be used for collecting seeds assumes critical importance. A pairwise mating system that provides N/2 matings out of N plants has been suggested. One progeny each from direct and reciprocal mating is saved to get seeds from a total of N progeny plants (Gale and Lawrence, 1984). This need not necessarily be the ideal system. For example, a trait recovery programme has been written for personal computers (Mansur *et al.*, 1990) which calculates the number of progeny that must be raised with a confidence level P to recover a specific

number of progeny (r) possessing a trait or genotype which occurs with known probability q. Calculations can be made for chosen values of p, r and q and the programme does fast and reliable estimation. A number of alternatives using the genetic principles of mating designs can also be thought of. The pollinating system, the extent of genetic variation within and between accessions, the traits for which variability needs to be preserved and the number of accessions to be regenerated in every cycle are a few important parameters to reckon with in deciding the strategy. In such decisions, the principle rarer the allele to be conserved higher the progeny size - is also to be kept in mind (Gale and Lawrence, 1984).

CONSERVATION OF GENE COMPLEXES

Finally, every gene bank has to seriously consider the possibilities of conserving populations and germplasm complexes. A realistic conservation activity would also take into account the 'utility index' of germplasm accessions in enhancing crop productivity (Arunachalam, 1988). Such yardsticks are necessary to keep the conservation process dynamic. In this context, the importance of 'species richness' consisting of the number of species and the richness of 'activity', each species undergoes in its target habitat when transferred from its original one, has been emphasized (Erwin, 1991). Intraspecific variation has been found to influence population gene diversity and hence the effective population size both in outbreeding and inbreeding species. This inference has been arrived at examining allelic frequency at isozyme loci in inbreeding and outbreeding plant species (Schoen and Brown, 1991). Crop variety mixtures have been found to fare well compared to their individual components particularly in marginal conditions varying in time and space (Jiggins, 1990). The possibility of enriching diversity by farmers growing such mixtures over time has been pointed out. In unmanaged pastures, deliberate incorporation of variability has been observed to be beneficial. Reinforcing physiological effects resulting in efficient utilization of growth factors have been postulated in favour of cultivar mixtures (Stutzel and Aufhammer, 1990). High build-up of parastic complexes would be dampened by systematic use of mixtures.

Viewed in the above background, and taking, other constraints on germplasm banks into account, there seems to be scientific logic for conserving germplasm complexes when the need arises. The opinion against conservation of 'mass reservoirs' in preference to maintaining individual components though lacking in diversity, would need critical review. Nevertheless, caution is needed to be conscious that genetic identity of individual components of a population or germplasm com-

plex would be lost over time or new genetic variability could be generated in open and often cross pollinated species. The horizon of germplasm conservation which includes the major component of evaluation is thus much wider and fast expanding with increasing information input. This could be a reason why the question, 'What genetic and germplasm stocks are worth conserving?' (Goodman, 1990) is being repeatedly asked. Though there is no unique answer, possible solutions from intensive research in light of summation given above can only be expected to feasibly converge.

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