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Genetic diversity and conservation and utilization of plant genetic resources

V. Ramanatha Rao* & Toby Hodgkin
*Senior Scientist (Genetic Diversity/Conservation), IPGRI-APO, Serdang, Malaysia and Principal Scientist, GRST, IPGRI, Rome, Italy (*requests for offprints; E-mail: v.rao@cgiar.org)*

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Abstract

Biodiversity refers to variation within the living world, while genetic diversity represents the heritable variation within and between populations of organisms, and in the context of this paper, among plant species. This pool of genetic variation within an inter-mating population is the basis for selection as well as for plant improvement. Thus, conservation of this plant genetic diversity is essential for present and future human well-being. During recent years, there has been increasing awareness of the importance of adopting a holistic view of biodiversity, including agricultural biodiversity, conservation for sustainable utilization and development. These principles have been enshrined in the Convention on Biological Diversity and the Global Plan of Action of the Food and Agriculture Organization of the United Nations. The emphasis is now to understand the distribution and extent of genetic diversity available to humans in plant species, so that the genetic diversity can be safely conserved and efficiently used. It is generally recognized that plant genetic diversity changes in time and space. The extent and distribution of genetic diversity in a plant species depends on its evolution and breeding system, ecological and geographical factors, past bottlenecks, and often by many human factors. Much of the large amount of diversity of a species may be found within individual populations, or partitioned among a number of different populations.

A better understanding of genetic diversity and its distribution is essential for its conservation and use. It will help us in determining what to conserve as well as where to conserve, and will improve our understanding of the taxonomy and origin and evolution of plant species of interest. Knowledge of both these topics is essential for collecting and use of any plant species and its wild relatives. In order to mange conserved germplasm better, there is also a need to understand the genetic diversity that is present in collections. This will help us to rationalize collections and develop and adopt better protocols for regeneration of germplasm seed. Through improved characterization and development of core collections based on genetic diversity information, it will be possible to exploit the available resources in more valuable ways.

Introduction

Biological diversity may be defined as the variation present in all species of plants and animals, their genetic material and the ecosystems in which they occur. Diversity can occur at three levels: genetic diversity (variation in genes and genotypes), species diversity (species richness) and ecosystem diversity (communities of species and their environment). The importance of biodiversity for humankind has been well recognised in the recent decades and many would argue that diversity is essential for allowing sustainable development of various human activities. Biological diversity can enable social and economic systems to flourish in ways that allow the poorest to meet their food and nutritional needs and retain the cultural diversity of countries throughout the world (Shiva, 1994). The biological resources of each country are important, but not all countries are equally endowed, and cooperation between countries is needed for effective conservation and use of our global biodiversity.

During the past few years there has been increasing awareness of the importance of adopting a holistic view of biodiversity, including agricultural biodiversity, and of linking conservation with sustainable utilization and development (Arora, 1997). For many, this began with the establishment of an *ad hoc* 'Working Group of Experts on Biological Diversity' in June 1987 by the United Nations Environment Programme (UNEP). This process led to the organization of the United National Conference on Environment and Development (UNCED) in June 1992, at Rio de Janeiro, Brazil. At UNCED, the Convention on Biological Diversity (CBD), and the action plan Agenda 21 were developed which have formed the basis for discussion and policy implementation since then. As a follow up to these developments, the International Technical Conference (ITC) was organized by the Food and Agriculture Organization of the United Nations (FAO) in July 1996 in Leipzig, Germany, focusing on agrobiodiversity. The work of preparing for this conference generated valuable information, at global, regional and national levels, on overall status of conservation, utilization, monitoring and management of plant genetic resources for food and agriculture (PGRFA). The conference adopted a 20-point Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (GPA), which was agreed to by over 150 countries participating in the conference (FAO, 1996a, b). There have been several follow-up meetings to the CBD and GPA and most of our on-going and future activities on PGR at global, regional and national levels will depend on how we can best plan, integrate and coordinate the activities on biodiversity conservation and use.

Plant genetic resources are among the most essential of the world's natural resources and during the last 2–3 decades, major advances have been made in conserving them (Frankel and Bennet, 1970; Frankel Hawkes, 1975; Holden and Williams, 1984; Plucknett et al., 1987; Watanabe et al., 1998; Ramanatha Rao et al., 1999). However, plant genetic resource conservation merits far greater attention than it is now receiving. Over the past few years, there have been some welcome developments, such as increasing efforts to develop improved *in situ* conservation methods which would permit dynamic conservation of plant populations (Jarvis, 1999; Sthapit and Jarvis, 1999). This is of particular importance for the effective maintenance of wild relatives of crops. It has been accompanied by a recognition that, in fact, *in situ* and *ex situ* conservation methods are complementary and the method chosen should depend on the species concerned and such factors as its distribution and ecology as well as the availability of resources in areas in which it occurs.

The general trend of the past decades has been the release and cultivation of improved cultivars of many major and minor crop species. These cultivars tended to be uniform. They are usually derived from a limited number of elite lines, which are often used in the production of many cultivars, resulting in an increasingly narrow genetic base for the crop. This, together with large-scale cultivation of such genetically uniform cultivars, has increased the genetic vulnerability of many major agricultural crop species, often with disastrous consequences. An often quoted-example is the Irish potato famine of 1840s, when the potato crop in Ireland was virtually wiped out as the potato varieties grown then had no resistance to the leaf blight disease. Similarly the outbreak of rice brown spot disease in Bengal area in 1943, aggravated by typhoons, contributed to serious famine in India (Council, 1972). In 1970, as a result of southern corn leaf blight, corn production decreased by about 25% in the southern states of the USA (Anon, 1973). However, in many of these cases, public and private plant breeders had access to genetic diversity and were able to produce resistant material within a relatively short time. Vulnerability due to increasing uniformity continues and several potential disasters may be brewing right now. For example, several important traditional crops of Oceania are highly threatened due to their narrow genetic base (Lebot, 1992). In 1993, taro leaf blight destroyed about 95% of the taro crop in Samoa, where it is the major staple food. The vineyards in California are being invaded by new biotype of phylloxera, the aphid relative that affects the root system of vines. Since more than 70% of wine grapes in Napa and Sonata counties are grafted on susceptible rootstock, the grape crop is seriously threatened and the possibility of the spread of the disease is considered very likely (Granett et al., 1991). Given this situation, it is necessary to broaden the genetic base of crop plants requiring access to a large amount of genetic diversity. Although, the results of some surveys (Brown, 1983; Chang, 1994; Smale, 1997) indicate that the genetic base of several important crops has begun to increase over the years, breeding programmes of many important crops continue to include only a small part of genetic diversity available and the introduction of new and improved cultivars continues to replace indigenous varieties containing potentially useful germplasm. In fact, genetic diversity can be seen as a defence against problems caused by genetic vulnerability. Traditional farmers built this defence into the genetic structure of landraces through selection over many generations and it may be necessary to introgress such defence mechanisms into modern cultivars to make them sustainable (Martin et al., 1991; Chang, 1994; Kannenberg and Falk, 1995).

Effective conservation of plant genetic resources requires a sound scientific and technical basis. An understanding is needed of the conservation methods that can be used and the ways in which they can be deployed. Central to any effective conservation programme must be a clear understanding of the extant genetic diversity in the species of concern – its structure and distribution in nature and in the material conserved, either *ex situ* or *in situ* (Allard, 1988; Hamrick and Godt, 1990; Hamrick et al., 1991; Hamrick, 1993; Hamrick et al., 1993; Hamrick and Godt, 1997). In this paper, we describe some of the factors affecting the distribution of genetic diversity and the ways in which information on genetic diversity is necessary in the conservation and use of plant genetic resources. The emphasis in this paper is on crop plants and their wild relatives although, relevant information from other useful plant species is included. Thus, we do not deal here with forest genetic resources, conservation of, which may be more complex than crop species. The available literature is reviewed, and some research needs are identified.

The extent and distribution of genetic diversity

Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety, or population of a species (Brown, 1983). It results from the many genetic differences between individuals and may be manifest in differences in DNA sequence, in biochemical characteristics (e.g. in protein structure or isoenzyme properties), in physiological properties (e.g. abiotic stress resistance or growth rate) or in morphological characters such as flower colour or plant form. Four components of genetic diversity can be usefully distinguished; the number of different forms (alleles) ultimately found in different populations, their distribution, and the effect they have on performance and the overall distinctness between different populations. The variation that underpins genetic diversity arises from mutation and recombination. Selection, genetic drift and gene flow act on the alleles present in different populations to cause variation in the diversity in them. The selection can be natural or it can be artificial, as is the case with much of the variation present in crop species (Suneson, 1960; Frankel, 1977; Nevo et al., 1984; Brown, 1988; Hamrick et al., 1992).

It is generally accepted that the genetic variation in plant populations is structured in space and time (Loveless and Hamrick, 1984). The description of the extent and distribution of the different aspects of genetic diversity in a species, and of the way in which it is structured, is an essential prerequisite to determining what to conserve, and where and how to conserve it. To date, most conservation efforts, either *in situ* or *ex situ*, have proceeded with little information on the genetic diversity that was being conserved and there is an urgent need to remedy this situation. The development of such improved descriptions involves not only describing the variation observed, but also identifying the major factors likely to affect the genetic structure of plant populations and determining the effect they have on the amount of variation in a population and the distribution of alleles in it. Such factors include climatic, edaphic and biotic ones as well as those specific to the populations (e.g. population size, selection), or to the species (e.g. ploidy, breeding system, linkage).

Genetic diversity is the basis for survival and adaptation and makes it possible to continue and advance the adaptive processes on which evolutionary success and, to some extent human survival, depends. Survival and adaptation can be viewed in terms of time, space and fitness. Fitness includes adaptation, genetic stability and variability. The process of extinction can be due to biotic or abiotic stresses, caused by factors such as competition, predation, parasitism and disease, or to isolation and habitat alteration due to slow geological and climatic change, natural catastrophes or human activities. Given these threats, it is essential that the genetic diversity in plant genetic resources be properly understood and efficiently conserved and used.

Ecogeographic factors

Geographic differences in the distribution of genetic diversity are extremely common. Populations may differ with respect to all aspects of diversity and show variation in the number of alleles, the identity of those alleles and the effect they have on the characteristics in the population. The breeding system of the species is very important in determining the differences between populations from different geographic locations. Outbreeders often show much more gradual changes between populations (e.g. Lanner-Herrera et al., 1996) reflected often in changes in allele frequency often of a clinal nature (for example, sorghum in W. Africa see, Morden, Doebley et al., 1989). In contrast, self-pollinated species show much greater differences between populations often with quite different alleles in different populations (Tachida and Yoshimaru, 1996).

In fact, geographic variation in distribution is nearly always impossible to separate from ecologically determined variation. Different geographic locations nearly always differ with respect to some potentially significant ecological characteristic (such as latitude, altitude, temperature, and moisture availability). For this reason they are often considered together as ‘ecogeographic factors’.

In general, under natural conditions, there is a close relationship between the morphological and the physiological traits of plants and of habitats in which the traits have evolved and are expressed. So much so, that the habitats may be defined in terms of the characteristics of the plant populations naturally occupying them (Bennett, 1970). Adaptive genetic variation, which is generally quantitative and responsive even to small habitat differences, often reacts with great sensitivity. Many studies have clearly demonstrated that there is a clear association between population characteristics and the environments in which they occur (Aston and Bradshaw, 1966; Al Hiyaly et al., 1993). The work of Nevo’s group (Nevo, 1978; Nevo, 1979; Nevo et al., 1981; Nevo et al., 1983; Nevo, 1990; Nevo et al., 1991, see also below) also emphasizes the role of ecological factors in determining the extent and distribution of genetic diversity in crop wild relatives. However, this is not universally the case and there are cases where plasticity has apparently been sufficient to allow rather genetically similar populations to occur in widely differing environments (McNeilly, 1997).

In practical terms, ecotypic differentiation in crop plants affects many traits such as the relative rates of development, resistances to biotic and abiotic stresses, edaphic responses and response to soil fertility, and adaptation to cultivation, irrigation and harvesting methods as well as quality differences (Bennett, 1970; Bradshaw, 1984). Most important is the fact that the local crop races are the consequences of long periods of interaction between the environment and genetic systems (IPGRI, 1994; Brush, 1995). Their outstanding characteristic is climatic and edaphic adaptation (Harlan, 1975a, b). Along with breeding systems, which affect significantly the factors determining the genetic composition of populations, it has been demonstrated that several ecological pressures affect the distribution of intrapopulation variability and the accumulation of genetic differences between two populations (Baatout et al., 1990). Selection for ecological adaptation has also been identified as a major force in the accumulation of differences in originally identical wheat populations grown in different parts of France (Goldringer et al., 1998). After 10 years, southern populations flowered noticeably earlier than Northern ones. Disease resistance properties had also changed in response to differences in the disease present in the different areas.

In crops, geographic factors that reflect social and political differences, may be as significant as ecological factors in determining the distribution of genetic diversity. In many studies, using genebank accessions, the country of origin appears to be an extremely important factor in determining the distribution of genetic diversity (Allard et al., 1972; Kahler and Allard, 1981; Saghai-Maroof et al., 1990). Spagnoletti Zeuli and Qualset (1987) were able to identify accessions originating in Italy but collected in Egypt on the basis of an analysis of diversity in a world collection of durum wheat. This may reflect the tendency of materials to circulate within a country to a greater extent than between different countries. In wild materials the picture may be different. Thus, levels of allozyme diversity in populations many wild species are not always correlated with political boundaries (Levin, 1977; Yeh and O’Malley, 1980; Wendel and Parks, 1985).

The characteristics of crop plants are the product of thousands of years human management and it is not surprising that socio-economic factors have been identified as of great significance in determining the extent and distribution of diversity in many crops such as potato in Andes (Brush, 1991), maize in South America (Quiros et al., 1990), and Mexico (Louette et al., 1997) rice in Vietnam (Trinh et al., 1995). Different communities or different sections of a community often keep different amounts of diversity and different types of materials. Thus, different communities maintain different types of taro materials in China and high and middle-income groups maintain more intra-crop diversity in some Nepalese communities than lowincome groups (Rana et al., 2000). Local communities often continue to maintain very large amounts of diversity as an essential element of their practices. Thus, Colombo (1997) reported that the amounts of diversity of RAPD and AFLP molecular markers found in a single field of cassava from Brazil were comparable to that found in the world collection maintained at Centro Internacional de Agricoltura Tropical (CIAT), Colombia.

The amounts of diversity found in geographically or ecologically marginal areas of a crop or species may be very much reduced. This can be attributed to small population size, reduced gene flow, and historical factors and has been observed in *Limnathes alba* (De Arroyo, 1975), tomato (Rick et al., 1977) and taro (Yen and Wheeler, 1968; Yen, 1991). Such populations may also possess unique genes conferring resistance to stress, or disease. Thus, rice accessions from mountainous areas of Nepal have the greatest cold tolerance yet found in the crop, although the number of different varieties grown by the communities is much less than that grown by communities living at middle altitudes less subject to cold stress (Sthapit and Witcombe, 1998).

Breeding systems

The breeding system of a species profoundly affects allele distribution. The mating system, floral morphology and mode of reproduction all affect significantly the extent and distribution of the genetic diversity (Loveless and Hamrick, 1984). These effects are expressed both through geographical parameters and through factors under genetic control (Brown, 1990). Despite the central role of breeding systems, knowledge of them in particular species under investigation is often limited to a broad generalisation that the species is an outbreeder or inbreeder (Ellstrand, 1984; Jain et al., 1989; Goodell et al., 1997). Wild species often possess breeding systems different from those of their crop relatives and therefore raise different problems with respect to maintenance and regeneration in genebanks.

Information about the mating system of a species can provide insight into genotypic distribution in natural populations. Outcrossing helps plant populations maintain high levels of genetic diversity. Coniferous forest tree species are outbreeding, wind pollinated and often highly variable. The selfed progeny of most outbreeding species displays significant inbreeding depression in the form of decreased seed set, germination, survival and growth (Perry and Knowles, 1990). As a general trend, inbreeding and outbreeding species may not only differ in overall levels of genetic diversity (Miller and Tanksley, 1990), but they may also differ in the amount of within and between population variances of genetic diversity (Schoen and Brown, 1991). Inbreeders exhibit greater population to population variation and are more variable in how genetic variation is structured. These are important considerations while developing strategies for germplasm collecting and maintenance of genetic diversity of plant genetic resources.

However, it is also known that sometimes the correlation between breeding system and genetic diversity may not be very strong (Clegg et al., 1992). This apparent contradiction is attributable to various other parameters (some of which are yet not very clear) have to be considered. This also confirms that there is still a need to survey the current information on the breeding systems of the wild relatives of crop species, which should be complemented by research where information is lacking or needs refining.

The importance of bottlenecks

When an outbreeding population passes through a bottleneck (i.e. new populations are established with small number of progeny plants), genetic variation declines in proportion to the severity of the bottleneck (Chakraborty, 1977; Maruyama and Fuerst, 1984, 1985; McCommas and Bryant, 1990; Bryant and Meffert, 1996; Robichaux et al., 1997). The smaller the size of a population and the longer it remains small, the more genetic variation it will lose. While all components of genetic diversity are affected by small population size, bottlenecks are predicted to have a larger immediate effect on allelic diversity than on heterozygosity (Nei et al., 1975). Large losses of heterozygosity are most likely if the recovery of a population to a large size is slow or delayed for several generations. Low levels of heterozygosity at one or more loci in a population or species have been interpreted as the result of a loss of genetic variation in a previous bottleneck (e.g. Bonnell and Selander, 1974; Parker, 1991). These predictions suggest that one can use observed losses in heterozygotes or number of alleles to make inferences about past bottlenecks in natural populations. However, because of the large sampling variance among loci, many loci might have to be sampled to detect differences in variation between populations. More over random linkage associations within populations and differential levels of inbreeding among populations will further inflate sampling variation.

The alleles at most risk during a bottleneck event are those with low initial frequency, which contribute little to average heterozygosity. So, the major initial consequence of a bottleneck is loss of rare alleles rather than loss of average heterozygosity. Measures of average genetic variation are useful in detecting the effects of small population size, even though individual loci may deviate from expectation. In small populations, the probability of inbreeding increases. In a random mating finite population, the probability of selfing should be 1/N, where N is the population size. In outcrossing populations, in the absence of self-incompatibility, there will also be some selfing, even with random mating (Crow and Denniston, 1988; Muona and Harju, 1989).

Within and between population diversity

Partitioning of genetic diversity within and between populations is an important factor to be considered in most conservation efforts, especially in the case of *in situ* conservation. Measures of genetic variation in space are strongly affected by correlation between subdivisions used for sampling (Cavalli-Sforza and Feldman, 1990), especially with regard to variation in gene frequencies across populations. Usually the variance of gene frequencies is standardized by obtaining the mean gene frequency. Under the model of isolation by distance (usually called the 'stepping stone' model), at the stationary state the correlation between the gene frequencies of two populations falls exponentially with the geographic distance between them. A method has been developed (Tachida and Yoshimaru, 1996), to compute identity coefficients of two genes in the stepping-stone model with partial selfing, which is often the case with outbreeding species.

Though a part of the differences in subsamples or subdivisions is the result of adaptation to different environmental niches, local gene statistical properties that reflect their limitation to the interval 0 – 1 and the sampling behaviour that occurs at reproduction (Crow and Denniston, 1988). The pattern of migration additionally imposes a spatial structure. Migration, dependent on the distance between the subdivisions generates a correlation between neighbouring populations.

Research needed on genetic diversity extent and distribution

There continues to be a substantial need for research on many aspects of the extent and distribution of genetic diversity. In respect to the genetic diversity in useful plant species, there is a particular need to explore the ways in which farmer management practices and ecological or geographic factors interact to determine population structure. Is the diversity found in crop populations still largely explicable in terms of ecogeographic factors and domestication events or have socio-economic, cultural and political factors had the greatest impact and largely minimized the significance of biological or edaphic factors? Research is also needed on such factors as the distribution of allelic variation within and between populations, particularly with respect to multi-allelic associations and to the significance of linkage disequilibrium in determining the importance of linkage and allelic associations in different crop species. Another key issue with direct practical implications for conservation management strategies is the extent and effect of introgression between crop species and their wild relatives (Harris and Hillman, 1989; Jarvis and Hodgkin, 1999).

Research is also needed on the distribution of allelic variation within and between populations, geographic patterns of variation as well as and genomically determined allelic associations in the crops and species concerned along with research on breeding systems. Such studies will provide valuable information on a number of practical issues of germplasm management, including the classification of accessions by known allelic constitution and the detection of redundancy in collections.

Molecular analysis of diversity

Molecular methods have become an essential part of most studies on genetic diversity extend and distribution and in the analyses of breeding system, bottlenecks and other key features affecting genetic diversity patterns. The studies may use RFLPs, RAPDs, AFLPs or SSRs. It is important, however, to understand that different markers have different properties and will reflect different aspects of genetic diversity (Karp and Edwards, 1995).

The results obtained from different molecular markers may themselves be quite different from those obtained by using biochemical markers such as isozymes or agromorphological characters. The dendrograms derived from cluster, isozyme variation and RAPD loci in cucumbers and melons were found to be dissimilar, though these disparities were consistent with differences in the pedigrees and other information about each accession and species. A lower coefficient of variation was attained in the estimation of genetic differences when using RAPDs compared to isozymes. The disparity between the marker analyses made may be related to the amount of genome coverage characteristic of a particular marker system in species and its efficiency in sampling variation in a population (Staub et al., 1997). For example, a comparison of genetic similarity matrices revealed that, if the comparison involved both the cultivated and wild soybean accessions, estimates based on RFLPs, AFLPs and SSRs are highly correlated, indicating congruence between these assays. However, correlations of RAPD marker data with those obtained using other marker systems were lower, may be because RAPDs produce higher estimates of interspecific similarities. If the comparisons involved cultivated soybean only, then overall correlations between marker systems were significantly lower. Within *Glycine max*, RAPD and AFLP similarity estimates are more closely correlated than those involving other marker systems are (Powell, 1996).

The studies with wild *Beta maritima* populations in the UK, using molecular markers, provided evidence of significant gene flow between populations although the evidence from isozymes suggested that there was little gene flow (Raybould et al., 1996). This was interpreted by the authors as evidence of selection for traits associated with the isozyme markers. Gene flow and introgression from cultivated plants may have important consequences for the conservation of wild plant populations, though gene flow from a crop to a wild taxon does not necessarily result in a decrease in the genetic diversity of the native plant. This was concluded in gene flow study using isozymes on cultivated beets (sugar beet, red beet and Swiss chard: *Beta vulgaris* ssp. *vulgaris*) and the wild taxon, sea beet (*B. vulgaris* ssp. *maritima*) (Bartsch et al., 1999).

Genetic diversity assessment methods vary in

- the way they resolve genetic differences,
- the type of data generated and
- the taxonomic levels at which they can be most appropriately applied.

Different approaches can give different results (Nesbitt et al., 1995) suggesting that the character or scope of variation examined by each marker may differ (Hodgkin et al., 2001). Bretting and Widlencher (1995) reviewed the available approaches and described the usefulness and limitations of different genetic markers for plant genetic resources management. They also discussed the use of different parameters for genetic diversity and differences that might result using alternative approaches. Differences in the diversity characteristics observed may reflect the amount of genome coverage characteristic of a particular marker system in species and its efficiency in sampling variation in a population (Staub et al., 1997).

In the last decade, various molecular marker systems have been developed and used although the use of RAPDs still seems to be most common, despite the weaknesses of this marker system (Karp et al., 1997; Karp, 2001). The number and nature of the accessions used are also very variable. This *ad hoc* approach has definite weaknesses. Firstly, it is very difficult to compare different marker systems and determine which are the best for what purposes. Secondly it is difficult to get a clear picture of how effective molecular markers are in helping us to understand the extent and distribution of diversity in crop genepools (Hodgkin and Ramanatha Rao, 2001).

Microsatellites have obvious advantages for crop species (e.g. ease of use, high levels of simply inherited variation) although they remain expensive and time consuming to develop for each and every crop and generic markers have not been identified. Comparative studies of different marker systems are needed to determine the relative merits of the different approaches for different crops, wild species or situations, in order to permit researchers to make appropriate choice of methodology. Indeed, in general, there remains a need to develop improved methodologies for studying and sampling genetic diversity in populations (Hodgkin et al., 2001).

Thus, it is likely that molecular methods are most useful for estimating such features as gene flow, genetic drift and degree of outbreeding, while other marker systems may be most useful for studying adaptive variation. Information thus generated using different markers can provide valuable information on a number of practical issues of germplasm management, including the classification of accessions by known allelic constitution and detection of redundancy in collections.

Role of genetic diversity data

The data on the extent, structure and distribution of genetic diversity is necessary for several purposes. Almost all of them have significant direct or indirect consequences on the conservation and use of genetic diversity. Some such roles of genetic diversity data are discussed below.

Taxonomy

Taxonomy provides the necessary underpinning for many aspects of management of genetic resources. It allows us to distinguish a set of species priorities for conservation because of their relationship to crops. It allows clear definition of the crop groups that should be considered and their boundaries. It also permits clear and unequivocal communication between conservationists allowing them to exchange material and to describe its properties on the basis of a shared understanding of identity. Taxonomy reflects an accumulation of differences in the genome between different organisms to an extent that they are recognized as different taxa. In cultivated plants taxonomy it is especially difficult (Harlan and de Wet, 1971) to an extent that it has even been suggested that different procedures are appropriate (Hanelt, 1988)

The basis for speciation is genetic diversity, adaptation and fixation of genes. While, traditionally taxonomy has been based on morphological characters, in the case of crop plants, data generated using molecular techniques for taxonomic inferences has provided new insights into the phylogeny and taxonomy of many plant groups.

The classical taxonomic treatments were based on comparison of many morphological characters. However, genetic information can considerably increase our knowledge of relationships. Thus, chromosome paring in polyploid hybrids represents comparison of synaptic ability of many, if not all, Mendelian loci on the genome. Studies of genetic diversity, genetic distance and divergence using techniques such as, arm ratios, differential staining, electrophoresis, immunochemical reactions, and more recently measurements of DNA content, DNA hybridization and RFLP studies, allow comparisons involving smaller amounts of total DNA present, based on DNA sequence similarity (Kimber and Yen, 1990). These may reflect fundamental relationships based on sequence similarity and divergence and hence provide a much more detailed analysis of taxonomic relationship.

A number of features of molecular techniques have been identified which make them particularly useful in systematic studies (Palmer et al., 1988). These include:

- Molecular techniques make it possible to analyse numerous characters, which are also independent, whereas morphological analysis provides fewer characters, often of dubious homology.
- Morphology is prone to considerable convergence while most DNA regions are less so and even if there is some convergence, the genetic basis of convergence in molecules is better understood. Thus the molecular approach is more objective.
- Molecular markers are relatively independent of the environment (Beckmann and Soller, 1986).

A further advantage has been the opportunity that organellar DNA studies can analyse maternal and paternal lineages separately. For example, it has been proposed that the use of chloroplast DNA (cpDNA) restriction fragment analysis provides numerous independent molecular characters that can often rigorously define monophyletic lineages (Sytsma et al., 1991; Gielly and Taberlet, 1994; Tsumura et al., 1996) and exhibit low intraspecific variation. The latter assumption was reviewed and it was concluded that, far from being rare, intraspecific cpDNA variation is relatively common (Harris and Ingram, 1991). The possible effects of this on phylogenetic reconstructions are yet to be fully understood, suggesting that cpDNA data should be used along with a wider consideration of the degree of intra-individual and intraspecific variation and of the mode of plastid transmission. There may be some serious questions as to its inheritance patterns, which can create problems of interpretation (Dong and Wagner, 1994). However, there is no doubt that the use of both nuclear and organellar DNA in taxonomic studies is providing very interesting new insights in a number of plant families with important crop plants. In the Brassicineae, relationships between species are now seen rather differently in the light of molecular studies which tend to confirm results from interspecies crossing experiments (Warwick and Black, 1993).

Not surprisingly, there have been a number of reports of discrepancies between analyses of DNA and morphology in the estimation of plant phylogeny. The discrepancies may arise as result of procedural problems or biological attributes of the organisms. The problems can arise from within both the morphological and the molecular aspects of the study (Sytsma, 1990). Both kinds of problems must be better understood to permit a more thorough synthesis of DNA and morphology for further refinement or resolution of taxonomic problems (Spooner et al., 1991). This is expected to greatly enhance our understanding of the components of biodiversity, which we wish to conserve.

Whatever methods are used (and there are strong arguments to suggest that information from both molecular and classical approaches is required), there is no doubt of the need for additional and substantial taxonomic work on many families of useful plants. There has been an absolute decline in the number of taxonomists in recent years and the discipline is significantly under-supported (Parnell, 1993). At the same time there are many important useful plant families where the taxonomy is very poorly defined which is particularly the case for tropical plant genera such as *Solanum* with 1500 species (Bohs, 1990), bamboos with about 1250 species (Wang et al., 1980a, b; Watanabe et al., 1994; Rao and Ramanatha Rao, 1999a, b). Without this knowledge even the simple task of deciding what groups or types should be conserved becomes more or less impossible.

Origin and evolution

An understanding of the origin and evolution of crop species greatly helps both in collecting and use of any plant species and its wild relatives. This is particularly important if effective use of secondary and tertiary gene pools (see Harlan and de Wet, 1971; Harlan, 1992) is to be made. Information from taxonomic studies, ecogeographic surveys and from work on breeding systems can all provide important perspectives on the origin and evolution of the target species and are thus essential.

Several authors have proposed that two plant species isolated by a chromosomal barrier can, via hybridization, give rise to new fertile diploid species that are partially reproductively isolated from both the parents. This mode of hybrid speciation, termed recombinational speciation by Grant (1981a, b), has been modelled genetically (Stebbins, 1957; Grant, 1981) and tested by experimental synthesis of new ‘hybrid’ species by Stebbins (1957) and others. The actual extent of this mode of speciation in nature is unclear. The detailed genetic information necessary to confirm or reject the ‘hybrid’ origin is often lacking (Rieseberg et al., 1990). All these approaches used so far have one limitation – genetic additivity in the putative hybrid species generally cannot be demonstrated, especially for the quantitative morphological traits (Gallez and Gottlieb, 1982; Rieseberg et al., 1990). The molecular phylogenetic data as well as studies of the geographical distribution of molecular markers have confirmed Stebbins’ assertions that hybridization is both geographically and phylogenetically widespread. It is not clear whether the hybridization events recorded in the evolution of many plant groups are an accidental consequence of the evolutionary history of those lineages, with little significance other than to befuddle taxonomists, or whether they are indicative of larger role for hybridization in the origin and evolution of species (Rieseberg, 1995). Nonetheless there are a number of classic cases where interspecific hybridization has been essential in the development of crop plants. These include both tetraploid (durum) wheat *(Triticum dicoccoides*) which occurs in both wild and cultivated forms and hexaploid (bread) wheat (*Triticum aestivum*) which occurs only as a cultivated species. *Brassica napus* (oilseed rape and swede) is another amphidiploid species (obtained from crossing *B. rapa* and *B. oleracea*) that exists only in the cultivated form.

Isozyme evidence has also been used to study origin and evolution of crop plants (Jensen et al., 1979; Doebley et al., 1983; Goodman and Stuber, 1983; Buth, 1984; Jarret and Litz, 1986; Jarret and Litz, 1986; Chung et al., 1991; Spooner et al., 1992). Isozymes were used to detect hybridization at the diploid level because allozymes that are present in each of the two parental species would be found in the their offspring (Gallez and Gottlieb, 1982). However, isozymes are biparentally inherited characters, and, using the genetic markers representing both the biparentally inherited nuclear genome and a uniparentally or clonally inherited cytoplasmic genome such as a chloroplast (cp) DNA or mitochondrial (mt) DNA is recommended. To confirm the hybrid origin of a taxon, it should combine the alleles of its two parents and have a cpDNA similar to one of its parents (see earlier discussion on cpDNA).

Studies of the origin and evolution of crops provides an understanding of the ways in which observed patterns of genetic diversity reflect the process of domestication. Studies of domestication patterns in beans (*Phaseolus* sp.) have resulted in the identification of a number of major geographically distinct diversity groups which show rather complex relationships and evidence of some gene flow between them (Gepts, 1998; Tohme et al., 1996). This type of information provides conservation workers with a basis for the development of collecting strategies and can help users identify materials likely to be of greatest value in different crop improvement programmes.

More detailed studies of domestication can also help in developing a better understanding of observed patterns of diversity. Genes associated with domestication traits such as seed size or dispersal characteristics are bound to have particular states and be invariate within the domesticated crops. Thus, there will be areas of the genome that are fairly uniform, where diversity is minimal. The variation and distribution of alleles at loci close to these areas may differ markedly from those in other areas of the genome, as may be the case for pearl millet (Poncet et al., 1998). Within species patterns of diversity may also reflect the domestication process, particularly when a number of more or less complex morphological types have developed following domestication (e.g. spring and winter cereals or the many different vegetable *Brassica oleracea* or *Lactuca sativa* types).

Selecting what to conserve

Taxonomic information and studies on crop evolution and domestication provide a basic underpinning of knowledge that supports the conservation process. In each of these areas, an analysis of patterns of variation provides an essential element. Genetic diversity studies make even more direct contributions in selecting what to conserve, and ensuring that the resources are well managed and used. By measuring the range of available genetic diversity within and between populations of any species we can adjust our collecting, evaluating and breeding strategies to obtain maximum variation from any given wild population (Morikawa and Leggett, 1990) and crop materials. Some of the ways in which this can be done are illustrated in the next sections.

For both inbreeding and outbreeding species, a useful sampling strategy begins with a survey of population diversity before making decisions about how populations will be represented in germplasm collections, or where *in situ* conservation activities should be located. In inbreeding species, such surveys are particularly important because diversity is often unevenly distributed between populations and some populations or areas may be particularly significant in terms of the amounts of diversity they possess. In outbreeding species, the emphasis is more likely to be on ensuring a good coverage of the range of types found (Schoen and Brown, 1993) Some examples include *Avena* populations in Canary Islands (Morikawa and Leggett, 1990), wild mandarin, *Citrus tachibana* (Mak.) Tanaka, in Japan (Hirai et al., 1990), and mangoes in Indonesia (Bompard, 1995).

Of course, studies on the extent and distribution of genetic diversity need to be combined with other information if we are to realize their full value. They need to be integrated with information on habitat, degree of threat and physical and human geography. For example, signs of recent colonization, such as location along the roadside, tend to be correlated with reduced diversity in *Avena barbata* (Jain et al., 1981) and *Phaseolus vulgaris* (Gepts and Bliss, 1988), but for many species such information is lacking. In such cases, information about genetic diversity of individual populations, derived directly from isozyme surveys or other sources, may be critical in guiding germplasm collecting.

Rather different approaches to the acquisition of genetic diversity data may be required for crops, forages and wild relatives. For forages and wild relatives, ecological information may be relatively more important than for crops and analyses of patterns of diversity using isozymes or molecular markers may be extremely effective (for a review of a substantial body of work using this approach see Nevo, 1998). However, in crops, socio-economic and cultural information may be as important as ecological data and some preliminary assessment of diversity may be possible on the basis of numbers of cultivars identified by farmers and their observed agromorphological characteristics (Louette et al., 1997; Bajracharya et al., 1999).

The extent to which variation has already been satisfactorily sampled in existing collections, or can be found in suitably protected areas, is a major issue in planning new collecting missions or other conservation activities. Analysis of data from characterization studies in genebanks along with data from ecogeographical surveys, including the information on observed variation, will greatly help. There is a need to examine the possibility of combining and using the ecogeographic survey data and characterization data to plan germplasm collecting and management programmes to explore the complexity of problems involved and develop better strategies (Guarino, 1995; Maxted et al., 1995; Guarino et al., 1998).

As noted earlier, the use of molecular techniques in studying genetic diversity in recent years has contributed to better understanding of the extent and distribution of genetic diversity in a number of important plant species (Hodgkin and Debouck, 1992; Karp et al., 1998; Hodgkin et al., 2001). These methods, coupled with ecogeographic surveys provide information on species distribution as well as infraspecific diversity, permitting effective sampling of a particular region.

For example, genetic variation within and among 11 natural populations of *Pterocarpus macrocarpus* from different forest habitats of Thailand was examined using isozymes and a high degree of among-population differentiation was detected (Liengsiri et al., 1995). An east-west pattern of population grouping was observed. The prominent longitudinal differentiation of populations suggests that, for *ex situ* conservation, sampling should be done from a few trees from each population, but many populations should be sampled (analogous to sampling locally common genes). For making choices for *in situ* conservation, the authors noted, the study should take into consideration the range of distribution of the species, across the borders of countries. Using restriction fragment length polymorphisms (RFLPs) it was demonstrated that the probability of adding new genes to a tomato collection would be about 20 times higher by adding one accession of *Lycopersicon peruvianum*, a wild relative of tomato (Miller and Tanksley, 1990). Studies of the distribution of AFLP markers in Sri Lankan coconut populations have shown that as far as molecular diversity goes, the emphasis should be placed on collecting relatively large numbers of plants from a few populations since most of the observed diversity is within populations rather than between them (Perera et al., 1998; Perera et al., 1999). Similar studies using RAPD markers on coconut plantations of the South Pacific showed that approximately 60% of the observed diversity occurred within-populations, but this level varied between various populations and indicated the need for focusing on southern populations (Ashburner et al., 1997)

Molecular methods of analysing diversity are also important because they can refine existing investigation strategies**.** Developments in the area of extraction of DNA from dried specimens, such as herbarium material and fossils, will help us to better understand the patterns of genetic diversity and phylogenetic relationships (Pääbo and Wilson, 1991; Engeln, 1993). Methods that can be used in the field to assess within population diversity to facilitate sampling of maximum diversity can be particularly useful for perennial plants and those that do not normally produce viable seed. There are now a number of protocols which can be used to collect small amounts of leaf tissue or other material in the field for later analysis of diversity using molecular markers (see Adams et al., 1992, 1999; Muralidharan and Wemmer, 1994).

There remains a considerable debate about the relative value of information on genetic diversity from studies of agromorphological characters, isozymes or molecular markers. There is also debate about the use of different molecular markers (Powell et al., 1996; Karp et al., 1998) and increasing interest in using molecular markers which might give information about patterns of adaptive diversity (Karp, 2001).

Managing conserved germplasm

Information from genetic diversity studies and the use of genetic diversity analysis procedures should be of direct value in managing both *in situ* and *ex situ* conserved germplasm. So far, deliberate *in situ* conservation activities for crop plants and their wild relatives have been rather few. With the exception of the extensive studies on wild wheat carried out by Nevo and his colleagues (Nevo, 1998), very little investigation of plant genetic diversity has been carried out in such situations. Using morphological characters, isozymes and molecular markers, Nevo and colleagues have quite clearly established the ways in which diversity varies according to variation in soil type, available moisture and other environmental factors (e.g. Nevo et al., 1988; Nevo and Beiles, 1989; Owuor et al., 1997; Li et al., 1999). They have also shown the ways in which patterns of variation can change over time and alter both the numbers and types of alleles present. This work provides a basis for the development of various types of monitoring procedures for *in situ* conserved populations, but to our knowledge, these have yet to be tested and described in the literature.

Rationalisation of collections

Genetic diversity data provides information necessary to evaluate the extent to which a collection contains significant gaps in terms of the range of variation found in a species or significant redundancies, that is accessions with very similar characteristics. A concern with the frequent occurrence of duplicates (i.e. accessions with identical genetic characteristics) has often been voiced (FAO, 1996a, b) and Hintum and Visser, (1995a, b) discuss the various issues involved for seed genebanks. Molecular markers are likely to be increasingly used to identify near duplicates or very similar groups of accessions. Thus, Phippen et al. (1997) used RAPDs to analyse a group of morphologically similar ‘Golden Acre’ type cabbage accessions and concluded that reducing the accessions of the type to only 4 groups of accessions that would result in a loss of only 4.6% of the total variation in the group.

Duplicates in seed genebanks may not add substantially to seed genebank costs. However, plant genetic resources of many plant species have to be conserved maintaining live plants/trees in field plots or field genebanks. Maintenance of field genebanks is costly in terms of requirements for land, supplies, and labour. A high rate of loss of accessions often occurs in the collections due to biotic and abiotic stresses. For these reasons, there is obviously a limit to the number of accessions that can be maintained in field genebanks. Limiting the numbers to a manageable size needs to be carried out rationally and based on sound scientific principles (Nissilä et al., 1998). A major criterion is avoiding duplicate or near duplicates in the collection, which can, generally, reduce the size of field genebanks in many cases. The grouping of accessions according to their morphological similarities is the first and most important step to identify duplicate accessions. The verification of morphologically identical accessions with molecular markers can be made to confirm the similarity or otherwise (Connolly et al., 1994). Work that started with using RAPD markers to rationalize sweet potato collections (Saad et al., 1999) is now extending to use of microsatellites. DNA amplification fingerprinting (DAF) appears to be useful in sweet potato germplasm characterization and may be employed to identify duplicate accessions or for identifying core collections. DAF data may also be useful for facilitating the selection of parents for a breeding programme to ensure a broad genetic base (He et al., 1995).

Regeneration of germplasm seed

Even under optimum conditions, accessions held in *ex situ* storage will need to be regenerated after a number of years. The genetic diversity of the conserved material must be preserved during germplasm regeneration and this is more complex and difficult in the case of out-crossing species than in inbreeders (Porceddu and Jenkins, 1982). So, just as the breeding system is a significant factor in determining allele distribution and diversity in nature, it profoundly affects maintenance of diversity in collections. Outbreeding species possess higher diversity levels and contain less genetically differentiated populations than inbreeders (Hamrick and Godt, 1990). Information is required on the degree of outcrossing that occurs in order to devise appropriate regeneration strategies which will critically affect both the numbers of plants needed for regenerating populations and the required degree of isolation between accessions. Information on interspecific crossing relationships will also be required for determining isolation requirements.

Despite their importance, very little attention has been given to regeneration practices up to now. Information on the subject is minimal and most of the available information is scattered. Breese (1989) raised a number of questions on germplasm regeneration and emphasized the difficulty in making generalized recommendations. There are a number of questions that need answers before a rational strategy for retaining genetic diversity in *ex situ* collections can be formulated. These relate to genetic stability as well as loss of genetic diversity and require data from experiments using a range of different species as well as further theoretical studies. In particular, there is a need to support research to generate crop and species specific information on regeneration techniques, including issues such as isolation requirements, population sizes, and genetic drift, which will contribute to the formulation of better regeneration strategies to conserve genetic diversity (Ramanatha Rao, 1991; Engels and Ramanatha Rao, 1998).

Many aspects of conservation management would benefit substantially from improved understanding of the reproductive biology of different crops species and their wild relatives. A survey of breeding systems, along with further research on these systems and on aspects of reproductive biology, such as sterility mechanisms and incompatibility systems where these act to limit the use of genetic resources or the production of sufficient seed for storage, will assist in developing more effective germplasm conservation practices, both *in situ* and *ex situ*.

Improving use of germplasm

Characterization and evaluation

The accessibility of collections depends largely on the information available on them. Accurate passport and characterization data are the first requirements, but users of plant genetic resources, particularly plant breeders, have also emphasised the need for improved evaluation of accessions. Evaluation is a complex process and there is serious backlog in most collections. There are often very large numbers of accessions involved (frequently many thousands) and a number of the traits (e.g. resistances to biotic and abiotic stresses) are difficult to measure and subject to significant variation according to the environment in which they are measured. Improved evaluation procedures are needed and the use of augmented plot designs (Narain, 1990) provides one way of assessing large numbers of accessions in a single replicate with control plots that produces statistically satisfactory data. Core collections (see below) provide another way of concentrating evaluation resources for maximum results.

It is worth emphasising that both characterization and evaluation data provide an effective source of information for genetic diversity studies. The results can be used to help understand patterns of variaiton in crop species and to identify groups of accessions with high diversity or with shared characteristics. Analyses of agromorphological variation in sesame (Hodgkin et al., 1999) were used to develop core collections in both India and China. In China, there was an extremely close association of variation with specific agro-ecological zones although the associations between agroecological zone and diversity pattern were much less clearly developed for Indian sesame. Weltzien and her colleagues (Weltzien, 1989; Weltzien and Fichbeck, 1990) were also able to use agromorphological variation to identify patterns of variation in Syrian landraces of barley and to show how important moisture availability was to the way in which variation was distributed in the crop in Syria.

Core collections

Even where passport, characterization and evaluation data of germplasm samples of a crop or wild species are available, large numbers of accessions make it difficult to choose the most promising ones with which to work. One approach to this problem is the development of core collections. A core collection is intended to contain, with a minimum repetitiveness, the genetic diversity of a crop species and its wild relatives (Frankel and Brown, 1984; Brown, 1989a,b). It was envisaged that such collections, which would contain approximately 10% of the collection, or 2000–3000 accessions, whichever is the smaller, would provide the starting material for breeders in search of new variation or specific characters and research workers investigating diversity.

The concept of the core collection appears to offer a number of potential benefits to users of genetic resources. Plant breeders would have a manageable number of accessions to use in the search for new characters or character combinations and a structured way to evaluate whole collections. Other research workers would be able to concentrate studies on inheritance or test new technologies on a defined subset on which a substantial amount of data would be collected. More practically, genebanks with limited resources would be able to maintain the core collection, a rationally chosen set of accessions of crop species at relatively low cost. There are now a number of published investigations of the ways in which core collections can be established (e.g. Hodgkin et al., 1995; Johnson and Hodgkin, 1999). Over 60 core collections were identified in a recent survey (C. Spillane, 2000, personal communication) in a wide range of different crops and wild relatives. There remain important issues to be addressed in ensuring that optimum procedures are used for developing core collections. These include the extent to which ecogeographic data can provide an adequate basis for the development of a core, the sampling strategy to be adopted (so that interesting traits with low frequency will be represented), the importance of the genetic structure of the crop or species concerned, and the ways in which procedures should be modified for crops with different breeding systems and for clonally propagated ones.

Conclusions

The conservation of crop genetic resources can be difficult to sell, but the stakes are high (Smith and Schultes, 1990). There is pressing need for all those who are interested in plant genetic resources conservation and use to be more involved in all the aspects of genetic diversity – to study, understand, enhance, conserve and use it. To do so, we need to understand the extent and distribution of diversity in species and ecosystems through appropriate research, field studies and analysis. Any conservation effort should be an approach that leads to integrated conservation – a balance of *ex situ* and *in situ* methods. There is a need to stimulate international cooperation or joint ventures on all aspects of plant genetic resources. Genetic diversity should be understood at all the three levels: at the level of species, at the level of genus and at the level of ecosystem. Additionally various interactions that affect allelic diversity and differences in allelic frequencies within and between populations need to be understood. We need to survey genetic diversity using all available methods of measuring, before identifying the areas and species to be conserved *ex situ* and *in situ*. Appropriate use of systematics, conservation biology and landscape ecology should be made to guide conservation, inventory, study and use of the biodiversity.

There remain many unresolved questions about the extent and distribution of genetic diversity in useful plant species. To what extent and in what ways are ecological factors important for the distribution of diversity in crops, forages or for their wild relatives? How do humans modify the underlying biological properties of the species in respect of different elements of diversity? What is the most useful combination of molecular, biochemical and agromorphological characters for the required understanding of the patterns of diversity? It is important that these are tackled in a systematic way and not through the continued accumulation of data in an almost random fashion that is often is the case. This will require cooperation between investigators, research centres and countries. In the light of increased use of molecular methods for studying plant genetic diversity, there is also the need to link the information on molecular variation to plant genetic resources management in a more meaningful way than it is presently done and this could be done on particular crop gene pools.

The major elements that confer value on genetic diversity and its organization are:

- the genetic integrity of evolved populations and taxa, or samples of these;
- the environments and ecosystems that support both the diversity and its structure, and
- its relationship with the ecosystem (Riggs, 1990).

The key to genetic conservation is maintaining and integrating these three elements. To achieve this we need to improve access to existing knowledge as much as possible, maintain genetic continuity and integrity wherever possible, and integrate and coordinate different conservation efforts.

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