GENETIC CHARACTERIZATION AND ITS USE IN DECISION MAKING FOR THE CONSERVATION OF CROP GERMPLASM

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Abstract
In this paper, we first present a brief update on the progress made in using genetic characterization to guide decision making for several conservation activities. Next, we focus on the attractive prospects offered by molecular characterization to enhance germplasm use, which is the ultimate purpose of conserving diversity of genetic resources.

Keywords
Genetic resources conservation, germplasm use, molecular markers

Introduction

Conservation of genetic resources entails several activities, many of which may greatly benefit from knowledge generated through applying molecular marker technologies. This is the case for activities related to the acquisition of germplasm (locating and describing the diversity), its conservation (using effective procedures) and evaluation for useful traits. In all, the availability of sound genetic information ensures that decisions made on conservation will be better informed and will result in improved germplasm management. Of the activities related to genetic resources, those involving germplasm evaluation and the addition of value to genetic resources are particularly important as they help identify genes and traits, and thus provide the foundation on which to enhance use of collections.

'Characterization' is the description of a character or quality of an individual [1]. The word 'characterize' is also a synonym of 'distinguish', that is, to mark as separate or different, or to separate into kinds, classes or categories. Thus, characterization of genetic resources refers to the process by which accessions are identified or differentiated. This identification may, in broad terms, refer to any difference in the appearance or make-up of an accession. In the agreed terminology of gene banks and germplasm management, the term 'characterization' stands for the description of characters that are usually highly heritable, easily seen by the eye and equally expressed in all environments [2]. In genetic terms, characterization refers to the detection of variation as a result of differences in either DNA sequences or specific genes or modifying factors. This genetic connotation will be used in this paper.

Standard characterization and evaluation of accessions may be routinely carried out by using different methods, including traditional practices such as the use of descriptor lists of morphological characters. They may also involve evaluation of agronomic performance under various environmental conditions. In contrast, genetic characterization refers to the description of attributes that follow a Mendelian inheritance or that involve specific DNA sequences. In this context, the application of biochemical assays such as those that detect differences between isozymes or protein profiles, the application of molecular markers and

the identification of particular sequences through diverse genomic approaches all qualify as genetic characterization methods.
Because of its nature, genetic characterization clearly offers an enhanced power for detecting diversity (including genotypes and genes) that exceeds that of traditional methods. Likewise, genetic characterization with molecular technologies offers greater power of detection than do phenotypic methods (e.g. isozymes). This is because molecular methods reveal differences in genotypes, that is, in the ultimate level of variation embodied by the DNA sequences of an individual and uninfluenced by environment. In contrast, differences revealed by phenotypic approaches are at the level of gene expression (proteins).

Using molecular characterization to make informed decisions on the conservation of crop genetic resources

Information about the genetic make-up of accessions helps decision making for conservation activities, which range from collecting and managing through identifying genes to adding value to genetic resources.
Well-informed sampling strategies for germplasm material destined for *ex situ* conservation and designation of priority sites (i.e. identifying specific areas with desirable genetic diversity) for *in situ* conservation are both crucial for successful conservation efforts. In turn, defining strategies is dependent on knowledge of location, distribution and extent of genetic diversity. Molecular characterization, by itself or in conjunction with other data (phenotypic traits or geo-referenced data), provides reliable information for assessing, among other factors, the amount of genetic diversity [3], the structure of diversity in samples and populations [4, 5], rates of genetic divergence among populations [6] and the distribution of diversity in populations found in different locations [7, 3].
A recent study on the genetic diversity of cultivated *Capsicum* species in Guatemalan home gardens compared the diversity present in an array of home gardens in the Department of Alta Verapaz with a countrywide representative sample of 40 accessions conserved *ex situ* in the national collection [8]. The results showed that home gardens of Alta Verapaz (H = 0.251) contained as much diversity as the entire national *ex situ* collection (H = 0.281). These results thus suggest that, (1) home gardens are indeed an extremely important resource for *in situ* conservation of *Capsicum* germplasm in Guatemala, and as such they should not be neglected; (2) if further collecting activities were to be undertaken, special emphasis should be given to collecting in Alta Verapaz; and (3) additional collecting in Alta Verapaz alone could disclose novel genetic diversity that is absent from the national collection.
Conservation of clonally propagated crops demands more complex and expensive procedures. If these crops are maintained on-farm, their existence is endangered by several factors, one of which being the introduction of alternative improved varieties. Conservation efforts need then to be based on solid knowledge of clonal diversity. This was the case for Abyssinian banana or ensete (*Ensete ventricosum* (Welw.) Cheesman) from Ethiopia, which was analysed with AFLP markers [9]. Of the 146 clones from five different regions, only 4.8% of the total genetic variation was found between regions, whereas 95.2% was found within regions. The results led to a reduced number of clones for conservation and indicated the existence of a common practice of exchange of local types between regions, which, in its turn, emphasized the need to collect further in different farming systems.
A study on taro (*Colocasia esculenta* (L.) Schott) genetic diversity in the Pacific, using SSR markers, showed that many of the accessions from countries of the Pacific region were identical to those of Papua New Guinea. This indicates that originally the cultivars may have been introduced throughout the region from Papua New Guinea [10] and that collection of taro genetic diversity could focus on Papua New Guinea alone.

Molecular characterization also helps determine the breeding behaviour of species, individual reproductive success and the existence of gene flow, that is, the movement of alleles within and between populations of the same or related species, and its consequences [11]. Molecular data improve or even allow the elucidation of phylogeny, and provide the basic knowledge for understanding taxonomy, domestication and evolution [12]. As a result, information from molecular markers or DNA sequences offers a good basis for better conservation approaches.

Management of germplasm established in a collection (usually a field, seed or *in vitro* gene bank) comprises several activities. Usually, such activities seek to ensure the identity of the individually stored and maintained samples, to ensure the safeguarding of genetic integrity and genetic diversity and to have the material available for distribution to users. These tasks are primarily a responsibility of gene bank managers and curators, and involve the control of accessions on arrival at the facilities, as well as their continuous safeguarding for the future through regeneration and multiplication. For all these routine activities, information about the genetic constitution of samples or accessions is critical and provides possibly the most important means of measuring the quality of the work being performed.

Börner *et al.* (2000)[13] analysed bulk seed of wheat accessions to test their genetic integrity after 24 cycles of regeneration and after more than 50 years of storage at room temperature in a gene bank. They found neither contamination nor incorrect manipulation effects such as mechanical mixtures, but did identify one case of genetic drift in one accession. The fact that IPK-Gatersleben gene bank (Germany) splits its germplasm samples into either almost or completely pure lines, i.e. accessions, is expected to have contributed to this very positive finding (J. Engels, personal communication, 2005).

However, in the same gene bank, a study examined the genetic constitution of rye accessions that underwent frequent regeneration. Results showed that (1) a significant number of alleles present in the original sample was lacking in the newly regenerated material, and (2) new alleles in the new material were not present in the first regeneration sample [14]. Thus, the use of molecular markers can quickly help check whether changes in alleles or allele frequencies are taking place.

Molecular information has been used to weigh the need for decreasing the size of germplasm collections, which otherwise would add costs to the long-term conservation of germplasm. For instance, Dean *et al.* (1999) [15] used microsatellite markers to analyse the genetic diversity and structure of 19 sorghum accessions known as 'Orange' in the USDA's national sorghum collection. They found two redundant groups (involving five entries) among the 19 accessions evaluated. They also found that much of the total genetic variation was partitioned among accessions. As a result, the authors concluded that the number of accessions held by the US National Plant Germplasm System (NPGS) could be significantly reduced without risking the overall amount of genetic variation contained in these holdings.

Markers were also helpful in examining genetic identities and relationships of *Malus* accessions [16]. Eight primer pairs unambiguously differentiated 52 of 66 genotypes in a study that calculated the probability of any two genotypes being similar at all loci analysed as being about 1 in 1,000 million. The results not only discriminated among the genotypes, but were also shown to be useful for designing strategies for the collection and *in situ* conservation of wild *Malus* species.

Selected molecular technologies render cost-effective and comprehensive genotypic profiles of accessions ('fingerprints') that may be used to establish the identity of the material under study. Simultaneously, these technologies can detect contaminants (and, in the case of material mixtures, contamination with introgressed genes from other accessions or commercial varieties), as well as the presence of redundant materials (or 'duplicates') [17]. Moreover, molecular data provide the baseline for monitoring natural changes in the genetic

structure of the accession [18], or those occurring as a result of human intervention (e.g. seed regeneration or sampling for replanting in the field). Whatever the case, analysis of molecular information allows the design of strategies for either purging the consequences of inappropriate procedures or amending them to prevent future inconveniences [19].
A small number of potential duplicates were identified in a core collection of cassava (*Manihot esculenta* Crantz) when isozyme and AFLP profiles were compared [20]. The core collection had been assembled with information from traditional markers, which proved to be highly effective for selecting unique genotypes. Molecular data were used for efficiently verifying the previous work on the collection and ensure minimum repetition. The taro core collection for the Pacific region was treated in a similar manner [10]. Thus, gene bank managers can easily realize the potential value of using molecular methods to support and possibly modify or improve a gene bank's operations.
A special and increasingly important role of genetic characterization is that of identifying useful genes in germplasm, that is, of maximizing conservation efforts. Because the major justification for the existence of germplasm collections is use of the conserved accessions, it is important to identify those valuable genes that can help develop varieties that will be able to meet the challenges of current and future agriculture.
Characterization has benefited from several approaches resulting from advances in molecular genetics such as genetic and QTL mapping, and gene tagging [21, 22]. Research in this field has led to the acknowledgement of the value of wild relatives, in which modern techniques have discovered useful variation that could contribute to varietal improvement [23, 24]. Knowledge of molecular information in major crops and species and of the synteny of genomes, especially conservation of gene order, has also opened up prospects for identifying important genes or variants in other crop types, particularly those that receive little attention from formal research.

Future trends

Most marker technologies target genomic regions, which are selectively neutral; some technologies, however, target specific genes. The neutrality of markers is suitable for most uses in germplasm conservation and management. However, when the interest of conservation lies specifically in the diversity of traits of agronomic importance, some questions remain about the markers' representativeness. In such a case, those markers able to detect functional diversity are more suitable for characterizing germplasm collections.
Germplasm in collections can undergo molecular characterization that is structural, meaning 'based on the building blocks of the DNA sequence', and functional, that is, based on the identification of genes and their functions. Such characterization permits access to the raw materials—the genes—for nearly all the objectives of today's and tomorrow's breeding programmes. The information gathered from structural characterization not only provides increased clarity on existing genetic diversity and its organization in individuals, but it determines sample and population organization that ultimately may form the basis for functional characterization.
The increasing number of sequencing projects has resulted in an increased opportunity to produce expressed sequence tags (or ESTs) to which gene functions may be assigned. Moreover, such projects are making possible the compilation of an enormous amount of sequence data that can be used to develop markers linked to specific genes, which, in turn, may help identify novel functional variation [25, 26].
In addition, the development of novel technologies continues. This usually means decreased costs—a very significant point for their application in the tasks of conserving genetic resources, which tend to involve large numbers of samples and to have difficulties in sourcing

needed funds. Other improvements involve increasing the throughput—both in number of markers analysed and in number of samples—and simplifying technologies.

New developments are also taking place in designing better approaches to access new and useful genetic variation in collections, namely, allele mining and association genetics. Allele mining focuses on the detection of allelic variation in important genes and/or traits within a germplasm collection [27]. If the targeted DNA (either a gene of known function or a given sequence) is known, then the allelic variation (usually point mutations) in a collection can be identified, using methods developed for the purpose [28].

Association studies of artificial progenies are an alternative to segregation analysis for identifying useful genes by correlation of molecular markers and a specific phenotype [29]. Association studies can be performed on a germplasm collection and also on other materials, as long as significant linkage disequilibrium (LD) exists, for example, breeding materials. It may be especially useful for those crops where appropriate populations for genetic analysis cannot be obtained or their production is too time-consuming [30]. It is also useful for those crops for which sequence information does not exist and is unlikely to be available soon.

The challenges ahead

The importance of the variation captured in genetic resources in allowing evolution and/or facilitating plant breeding has been long recognized. However, appreciating the variation held in collections is not sufficient. Conservation of genetic resources needs to go hand in hand with enhanced use of the conserved material. Identifying and making available the allelic variation that makes up the genotype and phenotype provides the groundwork on which genetic resources can be used in, for example, plant breeding.

The number of accessions held collectively by all CGIAR gene banks is estimated as being almost 600 000 [31]. Together with the collections established by national programmes worldwide, this number reaches almost 6 million [31]. Without doubt, these genetic resources collections, together with uncollected germplasm and that held *in situ* and on-farm, harbour abundant quantities of hidden allelic variants. The challenge is to unravel the mysteries of this variation so it can be used for the benefit of humankind.

Gene banks hold large numbers of accessions, particularly of staple crops. Modern improvements in equipment and procedures allow considerable sample throughput. This can be costly. However, the more a technology develops, the lower its costs will be per data point and per sample. Nevertheless, the higher the throughput used, the higher the number of data points obtained. This requires adequate equipment—for handling and storing—and expertise—for handling and analysing—to draw adequate results from the investment.

One possible avenue for ensuring broader benefits from molecular characterization is the establishment of international collaboration for particular crops. Although, currently, equipment and expertise cannot be readily available worldwide, characterization networks are possible. In addition to carrying out the laboratory work, such networks would also facilitate access to information, thus fostering closer links between curators, breeders and molecular scientists. At the same time, those countries with little expertise or equipment can make steady progress in both areas and, hence, can make better use of the genetic resources that they hold [32].

More and more, technologies have increased throughputs, which generally means the generation of progressively larger amounts of data. Such data should not languish unused. If gene banks equip themselves with the latest technologies, then they should be able to translate such data into scientific knowledge. To do so, they need not only laboratory technical expertise, but also need bioinformatics staff. This means that through their molecular work, gene banks may keep not only live plant materials but also DNA and data. Hence, the banks

may develop appropriate new roles as providers of genetic resources and their accompanying data in an array of forms. Such broadening of the gene banks' roles implies that their clientele will also expand from plant breeders to include molecular geneticists, molecular biologists and even bioinformaticists. The expanded range of roles may even lead to including activities related to phenotyping, a type of characterization beyond the traditional description of morphology and general field performance. Phenotyping is very much linked to the usefulness of good molecular characterization as, together, they form the basis of progress in modern genomics research [33].

Conclusions

The most important challenges in the near future are certainly the identification of useful variation (real or potential) in germplasm and its use in guiding conservation decisions. Knowing the presence of useful genes and alleles would help in making decisions on the multiplication of accessions and the maintenance of seed stocks when responding to an expected higher demand for materials. Such information may also help in making decisions on heterogeneous accessions, where only some genotypes may possess useful alleles. The gene bank curator may have to decide to maintain the original material as is and separate a subpopulation carrying the desirable alleles and give it new accession numbers and management protocols. This will facilitate germplasm use and add value to the collections.

Similarly, genotypes with known and interesting genes and alleles can be added to core collections to make them more useful to the user community. To promote use of the main collection, a core collection is developed to capture 75% to 80% of the representative genetic diversity. From the user's perspective, such a core collection will gain value when accessions having known genes are added, even if the general genetic diversity present in these few additional accessions is already present in the core collection.

Last, an extreme concept that could arise, based on the knowledge of the presence of valuable genes and alleles, is that of building collections based on traits. This is not a novel idea *per se*, but the initiative may develop into real once sufficient genomic results become available. Certainly, recent scientific advancements are drawing closer to this future.

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