Chapter 3: An introduction to plant germplasm exploration and collecting: planning, methods and procedures, follow-up

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Abstract

The significant developments that have occurred in information technologies and molecular genetics and genomics have had a direct and beneficial impact on germplasm exploration and collecting. The powerful information-management tools that are now available to collectors allow them to combine many different data sets and to extract much more practically useful and relevant information for the planning of collecting missions as well as the collecting itself. Furthermore, the political and legal situations regarding the status of and ownership of plant genetic resources have changed drastically over the past 15 years. The implementation of the Convention on Biological Diversity (in 1993), the International Treaty on Plant Genetic Resources for Food and Agriculture (in 2006) and the establishment of the Nagoya Protocol (in 2011) have had and continue to have a significant impact on matters related to the access and benefit sharing of plant genetic resources. Ethical considerations and awareness have also evolved and have become more important for individual scientists when collecting germplasm in foreign countries.

Introduction

Since the first edition of these Technical Guidelines in 1995, there have been significant changes in policy, science and technology that have had a direct impact on exploration and collecting plant genetic resources.

Current status

Molecular tools

The technological developments that have taken place in molecular biology and genetics over the past 10 to 15 years (particularly the enormous decrease in the price of genotyping) have revolutionized our understanding of the way genetic diversity is spread across populations, species and genepools. This, combined with the new power of information management (including bioinformatics), provides a much

This chapter is a synthesis of new knowledge, procedures, best practices and references for collecting plant diversity since the publication of the 1995 volume *Collecting Plant Diversity: Technical Guidelines*, edited by Luigi Guarino, V. Ramanatha Rao and Robert Reid, and published by CAB International on behalf of the International Plant Genetic Resources Institute (IPGRI) (now Bioversity International), the Food and Agriculture Organization of the United Nations (FAO), the World Conservation Union (IUCN) and the United Nations Environment Programme (UNEP). The original text for Chapter 3: An Introduction to Plant Germplasm Exploration and Collecting: Planning, Methods and Procedures, Follow-up, authored by J. M. M. Engels, R. K. Arora and L. Guarino, has been made available online courtesy of CABI. The 2011 update of the Technical Guidelines, edited by L. Guarino, V. Ramanatha Rao and E. Goldberg, has been made available courtesy of Bioversity International.

Dedication: This chapter is dedicated to the late Dr R. K. Arora, co-author of the original chapter, who passed away after a brief illness in March 2010.

better basis for the plant collector to understand the taxonomy of a crop genepool, the sampling strategy to be followed, the sites to visit for collecting and how to optimize the conservation of genetic diversity in collections. There are strategies on how the content of crop collections can be improved (Van Treuren et al. 2009). For example, an optimization strategy that builds on the concept of core collection has been proposed. It relies on hierarchically structuring the crop genepool and assigning a relative importance to each of its different components. The resulting optimized distribution of the number of accessions is compared with the actual distribution, which allows under- and over-representation within a collection to be identified. This proposed optimization strategy is applicable to individual genebanks, as well as consortia of cooperating genebanks, which makes it relevant for ongoing activities aimed at sharing responsibilities among institutions, such as A European Genebank Integrated System (AEGIS) (http://aegis.cgiar.org).

Because of the increased importance of the exchange of DNA samples for research, it is important to include this in the overall collecting strategy. This means that good-quality, properly sampled plant material will become increasingly important, as will knowledge of the considerable differences among species as to which organs or plant parts are the most suitable for DNA extraction. For a very recent and complete publication on collecting for DNA banking, see Gemeinholzer et al. (2010). This paper gives guidelines for all the tasks required for pre-DNA isolation of samples, from both plants and animals. It includes information about sampling strategies, methodological considerations for collecting different types of plant tissues, strategies for tissue preservation and DNA isolation in the field, relevant logistics and safety considerations in the field, labelling of samples and recording essential information, transportation practices from the field to the laboratory and necessary equipment. Further details can be found in Chapter 40 of these Technical Guidelines (on collecting DNA for conservation), and Prendini et al. (2002) provide an earlier overview of this particular field.

In 2004, Bioversity conducted an international survey on the application of DNA technology in their genebanks, and of the 243 respondents, 51 (20.9%) indicated that they store DNA. Almost all of the institutes that store DNA extract it themselves, using standard manual extraction protocols, and one-third of these use commercial kits for DNA extraction. De Vicente and Andersson (2006) list a number of the reasons institutes, including genebanks and other organizations that collect plant genetic resources for food and agriculture (PGRFA), store DNA, as well as which techniques they use to serve their clients.

Information technologies and tools

The increased availability and ease of use of GIS technologies and GPS receivers over the past decade mean that sophisticated spatial analysis can be used routinely to support collecting efforts. Guarino et al. (2002) provide a comprehensive overview of how spatial analysis of the geo-referenced data generated by the process of conservation and use of genetic resources, using GIS, can feed back to enhance and facilitate the process and, indeed, to add value to germplasm collections. GIS allows the extent and distribution of diversity to be analysed and, thus, in the context of this collecting manual, allows the following activities to be undertaken more efficiently:

- ecogeographic surveying: the process of collating information on the taxonomy, genetic diversity, geographic distribution, ecological adaptation and ethnobotany of a plant group, as well as on the geography, ecology, climate and human setting of the study area. Such surveys can allow the identification of areas that are likely to contain genetic resources with specific traits or taxa of interest, areas that are highly diverse, areas that would complement other areas in conservation efforts, areas that contain material that is currently missing or underrepresented in collections, and areas where the genetic resources in question are threatened with genetic erosion. (See also Chapter 14, of this update, on ecogeographic surveys.)
- fieldwork: including field aids and the timing of collecting missions
- the design, management and monitoring of in situ reserves (Monitoring activity, in particular, is of
 potential relevance in the context of these Guidelines.)

GIS tools allow one to carry out complex analyses combining different (spatially referenced) data sources and to generate maps. This facilitates the uptake of outcomes by responsible authorities and encourages the

development and implementation of conservation policies. Tools such as FloraMap (see http://gcmd.nasa.gov/records/CIAT_FloraMap.html) and DIVA-GIS (www.diva-gis.org) can be used to determine potential distributions, assuming that climatic variables are the principal drivers of the geographic spread of plant species. Examples of the successful application of these tools include the location of wild and rare potato species in Peru (Hijmans and Spooner 2001) and optimizing a collecting mission for a rare wild Capsicum species (*C. flexuosum*) in Paraguay (Jarvis et al. 2005). Spatial analysis using GIS can also be used to identify regions of particularly high species richness and diversity.

The general accessibility and use of the internet has also revolutionised the sharing of data on biodiversity, geography and the environment. Scheldeman and van Zonneveld (2010) have produced a comprehensive training manual cum technical guide on the spatial analysis of data on plant diversity and distribution. See also Chapters 15/16 and Chapter 19 of this update.

Gap analysis is a systematic approach that allows collectors to target areas that contain traits and taxa that are underrepresented in existing *ex situ* collections. This method has been developed for crop wild relatives (see http://gisweb.ciat.cgiar.org/GapAnalysis/?p=139). The tool is a joint product of Bioversity International, the International Rice Research Institute (IRRI) and the International Centre for Tropical Agriculture (CIAT). More recently, Maxted et al. (2008) used the gap analysis methodology to identify actual areas for the *in situ* conservation of crop wild relatives. Castaneda and Maxted provide an update in Chapter 42 of these Technical Guidelines.

Legal and policy changes

In the policy area, the developments that have taken place with regard to access and benefit sharing—particularly those resulting from the International Treaty on Plant Genetic Resources for Food and Agriculture (hereinafter "the Treaty") and a number of protocols and agreements related to the Convention on Biological Diversity—have also had a direct influence on the procedures required to obtain access to genetic resources for collecting and/or exchange. The most recent relevant instrument is the so-called Nagoya Protocol on access and benefit sharing that was agreed upon during the COP 10 meeting in Nagoya, Japan, and has been signed by 92 countries, to date.

Prior informed consent (PIC) needs to be obtained from the relevant authorities in a given country before a collecting mission can be conducted, and access (i.e., permission to transfer the germplasm outside the country) is granted on the basis of mutually agreed terms (MAT), which may include measures that cover sharing with the providing country any benefits that might arise from the commercialization and utilization of the germplasm.

For crops and species that are included on the Annex I list of the Treaty, there is a standard material transfer agreement (SMTA) that needs to be concluded before any germplasm can be legally sent and/or received. (For more details on this, see Chapter 2 of these Technical Guidelines, on legal issues.) For non-Annex I crops, a material transfer agreement, established by the national authorities, needs to be included. However, in some instances (as in Europe) it has been agreed to also distribute germplasm that is non-Annex I material from genebanks with an SMTA that includes a specific footnote on its non-Annex I status.

Because of the political changes that have taken place over the past 10 years or so (and which are expected to continue) with respect to access to genetic resources and benefit sharing, organizations like the centres of the Consultative Group on International Agricultural Research (CGIAR) and individual countries have changed or are in the process of adjusting their collecting procedures to the new requirements. For instance, some countries like the following have established national legislation on access and benefit sharing of PGRFA (the years mentioned in brackets relate to the year of publication of the relevant regulation or law; in the case of the Nordic countries these dates are the same as they refer to a regional Ministerial Declaration on Access and Rights to Genetic Resources adopted in 2003): the Philippines (2005) and a number of European countries, including Bulgaria (2002), Denmark (2003), Finland (2003), France (2006), Hungary (2004), Iceland (2003), Italy (2007), Malta (2004), the Netherlands (2002), Norway (2003), Portugal (2002), Russia (2001), Spain (2007), Sweden (2004) and Switzerland (1999).

The legal situation and status of plant genetic resources has drastically changed and widened over the past 15 years. In many countries, the rights and status of local communities have been recognized, and many individuals have become aware of the importance of their own contributions to genetic diversity. With this in mind, it is important for collectors to inform themselves about the ethical considerations related to genetic resources, which might also have an impact on local and indigenous people. Recently, ethical considerations regarding the collecting, research and use of agricultural biodiversity have been reviewed, and recommendations on ethical issues have been made (Engels et al. 2010). Whereas easy access to genetic resources for breeding purposes is important, there are international agreements and legal frameworks that have been developed to ensure adequate recognition of the contributions of local communities and traditional farmers in creating and nurturing these resources. Consequently, Engels et al. (2010) have applied the code of ethics of the International Society of Ethnobotany to plant genetic resources to create awareness among scientists and policymakers who are concerned with agro-biodiversity research and its potential impact on local communities. Collectors are encouraged to seriously consider the ethical principles presented in the paper and to integrate these principles into their personal ethical framework.

The analysed code of ethics recognizes the *inalienability* of indigenous peoples, traditional societies and local communities: i.e., the right not to have their traditional territories and the natural resources within them, including related practices, cultures and the associated traditional knowledge, affected by the introduction of new but foreign ideas, inventions, knowledge and other findings. Indigenous communities should have the right to decide whether or not to accept such an introduction. The idea of the inalienability of rights has existed for authors of creative works in the "moral rights" aspect of copyright law, as embodied in the concept of the "integrity" of a created work. And a similar principle is included in Article 20 of the UN Declaration on the Rights of Indigenous Peoples, which states that indigenous peoples have the right to maintain and develop their political, economic and social systems or institutions. An example illustrating this principle is the introduction of new crop varieties with "improved" characteristics, such as short-stemmed teff (*Eragrostis tef*) in Ethiopia. Such varieties were developed without considering the importance of teff straw as a major source of livestock feed during the dry season.

Any research programme aimed at traditional farmers must first assess the needs and requirements of these farmers and include the intended users of the new variety in decisions on the specific breeding objectives for any given crop. Participatory plant-breeding approaches, which are included in a number of development projects, address this principle squarely and are increasingly seen to result in better-adapted and more sustainable varieties (see also Chapter 18 of these Technical Guidelines, on: a participatory approach to collecting plant genetic resources and documenting associated indigenous knowledge in the field). Consequently, in the context of initiatives on participatory breeding and crop improvement that include the collecting of genetic diversity from local farming communities, it would make good sense to involve the local farmers in such collecting activities in a participatory manner.

The principle of equity is another key consideration in the Convention on Biological Diversity and is an important component of the benefit-sharing arrangements that are expected to be established between the researcher or collector and the local people when obtaining and subsequently using agro-biodiversity or local knowledge. It is also one of the principles in the Code of Ethics of the International Society of Ethnobotany (http://ethnobiology.net/code-of-ethics) and is included in the UN Declaration on the Rights of Indigenous Peoples (http://www.un.org/esa/socdev/unpfii/documents/DRIPS en.pdf): the recognition that indigenous peoples must be fairly and adequately compensated for their contributions to research activities and outcomes involving their knowledge. The FAO Code of Conduct for Germplasm Collecting and Transfer (https://www.fao.org/nr/cgrfa/cgrfa-global/cgrfa-codes/en) also includes the equitable sharing of benefits arising from the use of collected germplasm.

Future challenges/needs/gaps

Worldwide, the lack of sufficient and well-qualified human capacity has been reported and experienced in
areas that are of critical importance to the collecting of genetic resources, e.g., taxonomy, genetics and
ecology (FAO 2010). This is a serious challenge for global conservation efforts, and the organizations
connected with conservation at different levels must take action to correct the situation.

- Whereas for a (few) major crops it has been reported that a large part of their genetic diversity is present in *ex situ* collections, many other crops, particularly the so-called "neglected and underutilized" species, and the crop wild relatives are significantly underrepresented at the species level—and even more so at the level of genetic diversity within species (FAO 2010).
- We now have a better understanding of the extent and nature of gaps in *ex situ* collections than we did when the first version of these Technical Guidelines was written; however, the picture is still far from complete. The use of molecular data to improve our understanding on the extent, nature and distribution of genetic diversity, more detailed field surveys and better geo-referencing of accessions will be very helpful to more accurately identify gaps and redundancy within and among collections as well as in a given genepool as a whole (FAO 2010).
- As the linkages between *in situ* and *ex situ* conservation have not been well defined, at least from a legal perspective, and for many crop genepools clear conservation strategies are missing to guide genebanks and their curators, it is not always clear whether it is better to collect from farmers' fields or natural habitats and store the germplasm *ex situ* or to keep it *in situ*/on-farm.
- The potential role of DNA samples and the storage of sequence information for conservation purposes is not fully recognized or established, and this (among other reasons) seems to hamper genebanks in moving forward faster with this technology in the management and distribution of genetic resources.

Conclusion

As a number of the changes and technological developments mentioned above are addressed in more detail in other chapters of these Technical Guidelines, the reader is advised to read the chapters or sections in which these developments and technologies/tools/methods are more fully described. Due to the ever-increasing amount of information the scientific community is producing on many of the species we are interested in collecting, studying and/or using in improvement programmes, there is a danger of information overload, and consequently, it is important to be very clear about the objective of a given exploration or collecting effort and to use the available information to the benefit of that effort.

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Internet resources

A European Genebank Integrated System (AEGIS): http://aegis.cgiar.org

Diva-GIS: www.diva-gis.org

FAO Code of Conduct for Germplasm Collecting and Transfer: www.fao.org/nr/cgrfa/cgrfa-global/cgrfa-codes/en

FloraMap: http://gcmd.nasa.gov/records/CIAT_FloraMap.html

Gap Analysis: http://gisweb.ciat.cgiar.org/GapAnalysis/?p=139

International Society of Ethnobotany (ISE): http://ethnobiology.net

International Treaty for Plant Genetic Resources for Food and Agriculture: www.planttreaty.org

Nagoya Protocol on Access and Benefit Sharing: www.cbd.int/abs

Standard Material Transfer Agreement: www.planttreaty.org/content/what-smta

United Nations Declaration of the Rights of Indigenous Peoples (UNDRIP): www.un.org/esa/socdey/unpfii/documents/DRIPS en.pdf