CHAPTER 5

Methodologies for generating variability. Part 1: Use of genetic resources in plant breeding

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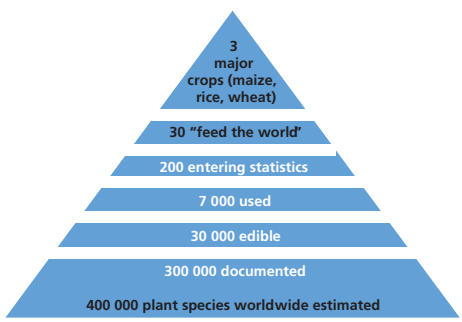
5.1 INTRODUCTION

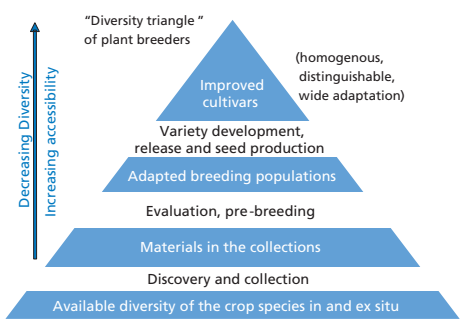
Both inter- and intraspecific diversity is declining in our present agricultural systems. Out of an estimated total of 30 000 (FAO, 1996a) to 50 000 (Sánchez-Monge, 2002) edible plant species, only 30 "feed the world", with the three major crops being maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) (FAO, 1996a; Figure 5.1). At the intraspecific level, plant breeding contributes to a diminution of diversity through development of narrow, elite breeding populations, selection of the 'best' genotypes, development of homogeneous cultivars, and promotion of a few, widely adapted varieties (Figure 5.2).

FIGURE 5.1
Use of crop species diversity in agriculture



Source: FAO, 1996

FIGURE 5.2
The diversity triangle of plant breeders



Source: FAO, 1996

However, the decline of inter- and intraspecific genetic variability among and within cultivated crop species bears with it several risks, including epidemics of pests and diseases due to greater genetic vulnerability; lack of adaptation to climate-change-related stresses; lack of genetic variation for specific quality traits; and reaching performance plateaus. A more efficient use of plant genetic diversity is therefore a prerequisite for meeting the challenges of development, food security and poverty alleviation (FAO, 1996b). Concrete aims of using plant genetic resources (PGR) in crop improvement are:

- to develop cultivars that are specifically adapted to abiotic or biotic stresses;
- to assure sustainable production in high-yielding environments through reduced application of agrochemicals and increased nutrient and water efficiency; and
- to open production alternatives for farmers through development of industrial, energy or pharmaceutical crops.

Methods of using PGR in crop improvement have recently been reviewed (Haussmann *et al.*, 2004). Major points will be summarized in this chapter, but for details and more examples, the reader is referred to the full review article. After the generalities concerning use of plant genetic resources (PGR) in plant breeding, this chapter will also consider more specific aspects of using plant genetic resources in participatory plant breeding, such as management of diversified populations and their potential contribution to *in situ* PGR conservation; the use of landraces as genetic resources for adaptation to stress environments, climate variability and climate change; and to better serve farmer's and end-user's diverse needs.

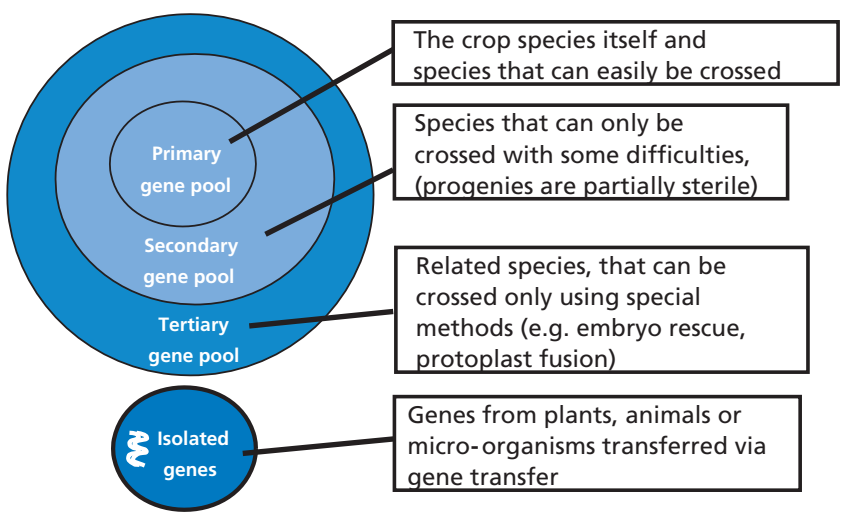
5.2 DEFINITION OF GENETIC RESOURCES FOR PLANT BREEDING

PGR can be defined as all materials that are available for modification of a cultivated plant species (Becker, 1993). PGR have also been considered as those materials that, without selection for adaptation to the target environment, do not have any immediate use (Hallauer and Miranda, 1981). According to the extended gene pool concept, genetic resources can be divided into primary gene pool; secondary gene pool; tertiary gene pool; and isolated genes (Harlan and de Wet, 1971; Becker, 1993; Figure 5.3). The primary gene pool consists of the crop species itself and other species that can be easily crossed with it. The secondary gene pool is composed of related species that are more difficult to cross with the target crop, i.e. where crossing is less successful (low percentage of viable kernels) and where crossing progenies are partially sterile. The tertiary gene pool consists of species that can only be used by employing special techniques, like embryo rescue or protoplast fusion. The fourth class of genetic resources, isolated genes, may derive from related or unrelated plant species, from animals or micro-organisms.

5.3 FACTS AND INFORMATION SOURCES

Worldwide, 1 308 gene banks are registered and conserve over 6.1 million accessions, including major crops, minor or neglected crop species, together with trees and wild plants. Of the 30 main crops, more than 3.6 million accessions are conserved *ex situ* (FAO, 1996a). Little information exists about documentation and availability of materials that are maintained *in situ*. Links to some of the most important organizations or networks dealing with PGR are listed in Box 5.1.

FIGURE 5.3
The extended gene pool concept for classification of PGR



Source: modified from Becker, 1993, and Harlan and de Wet, 1971.

BOX 5.1

Some important organizations and networks dealing with PGR.

- World Information and Early Warning System (WIEWS) on Plant Genetic Resources for Food and Agriculture (PGRFA) — http://apps3.fao.org/wiews/
- Consultative Group of International Agricultural Research (CGIAR) System-wide Information Network for Genetic Resources (SINGER) — www.singer.cgiar.org
- Bioversity International — www.bioversityinternational.org
- Germplasm Resources Information Network (GRIN) and the National Plant Germplasm System (NPGS) of the United States Department of Agriculture — www.ars-grin.gov/npgs/
- Mansfeld database — http://mansfeld.ipk-gatersleben.de/Mansfeld/
- The Information System on Genetic Resources (GENRES-International) — www.genres.de

5.4 DOCUMENTATION AND EVALUATION OF PGR

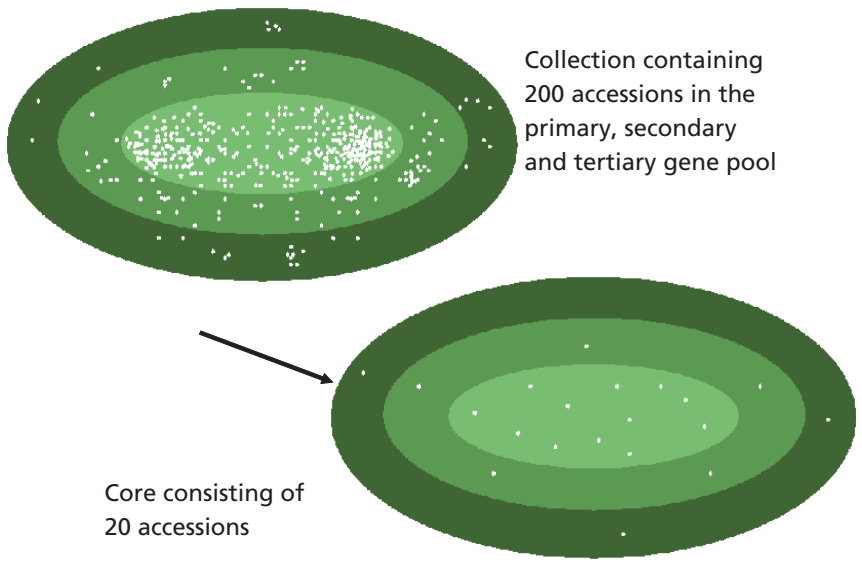
Gene bank accessions are described by passport and characterization data, and to a variable extent also by evaluation data. Passport data include serial number, taxonomic name, collection site, date of collection and donor institute. Additional notes can refer to seed viability, number and mode of regenerations or reproduction, and information about the distribution of the sample. Germplasm passport information exchange is facilitated by the internationally standardized list of multi-crop passport descriptors (FAO/IPGRI, 2001). Characterization data usually comprise scores for simple morphological traits like plant height, maturity date and thousand-seed weight. Evaluation data refer to agronomic traits like grain yield, grain quality, lodging and resistance to important pests and diseases as far as evaluated. Evaluation is a continuous process. Different people or institutions can be involved, including gene banks, breeders, pathologists or physiologists searching for or studying specific traits. Ideally, all data sets referring to an accession are stored in a central database and are made available to the public.

Systematic evaluation of germplasm conserved *ex situ* is facilitated through development of core collections. Initially, core collections were defined as a limited set of accessions representing, with a minimum of repetition, the genetic diversity of a crop species and its wild relatives (Frankel, 1984). In the context of an individual gene bank, a core collection consists of a limited number of the accessions of an existing collection, chosen to represent the genetic spectrum of the whole collection (Brown, 1995; Figure 5.4). Core collections render the evaluation process more efficient because repetition of similar entries is avoided (Hodgkin *et al.*, 1995; van Hintum *et al.*, 2000).

5.5 ACCESS TO PLANT GENETIC RESOURCES, EQUITABLE SHARING OF PROFITS AND BENEFITS, AND MATERIAL TRANSFER AGREEMENTS

The Convention on Biological Diversity (CBD) aims at the conservation and sustainable use of biological diversity, and an equitable sharing of profits and benefits generated by the use of genetic resources (www.cbd.int). One aim of the convention is to ensure recognition of the past, present and future contributions of farmers to the

FIGURE 5.4
The concept of core collection



conservation and development of genetic diversity (Swaminathan, 2002). To fulfil the convention, so called Material Transfer Agreements (MTAs) have been developed. The Standard MTA (SMTA, www.cgiar.org.cn/pdf/SMTA_English.pdf) protects the genetic resources of plant species listed in the Annex 1 of the International Treaty on Plant Genetic Resources in Food and Agriculture (www.fao.org/ag/cgrfa/itpgr.htm#text) against intellectual property rights and assures continuous and free availability. A special paragraph deals with the equal sharing of benefits (Figure 5.5).

MTAs from other institutions may refer to restricted plant materials, and in this case the user has to agree to use the material for research only; not to distribute or commercialize the plant material or derived materials; and to take all reasonable precautions to prevent unauthorized propagation of any of this material or derived plant materials.

5.6 METHODS OF USING GENETIC RESOURCES IN PLANT BREEDING

After identification and acquisition of potentially useful PGR, there are generally four ways of using those genetic resources in plant breeding (Simmonds, 1993; Cooper, Spillane and Hodgkin, 2001; Figure 5.6):

- introgression, which involves the transfer of one or few genes or gene complexes (chromosome segments) from a genetic resource into breeding materials;
- incorporation (also named genetic enhancement or base broadening) describes the development of new, genetically broad, adapted populations with a new range of quantitative variation and acceptable performance level;
- pre-breeding, which refers to more basic research activities with the goal of facilitating use of 'difficult' materials; and
- gene transfer.

Sometimes, the categories cannot be clearly separated one from another.

FIGURE 5.5
Some key clauses of the Standard Material Transfer Agreement (SMTA)

The Recipient may utilize and conserve the material for research, breeding or training purposes.

The Recipient shall not claim any intellectual property or other rights that limit the facilitated access to the Material provided under this Agreement, or its genetic parts or components.

In the case that the Recipient commercializes a product that is a Plant Genetic Resource for Food and Agriculture and that incorporates Material as referred to in Article 3 of this Agreement, and where such Product is not available without restriction to others for further research and breeding, the Recipient shall pay a fixed percentage of the sales of the commercialized product into the mechanism established by the Governing Body for this purpose, in accordance with Annex 2 to this Agreement. …

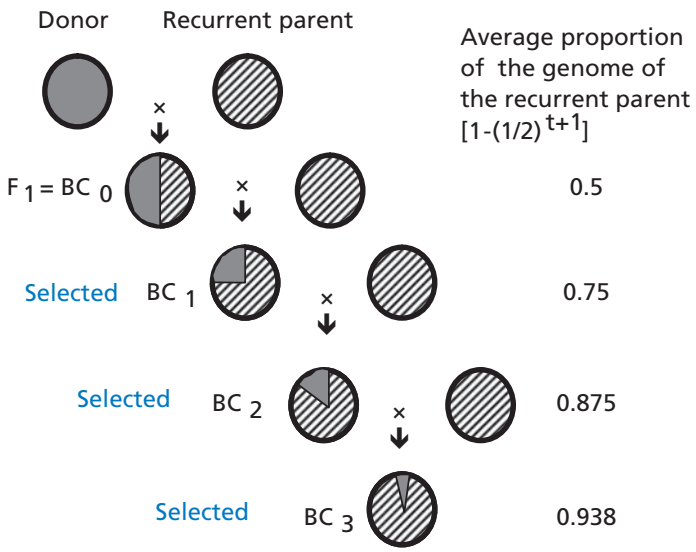
FIGURE 5.6
Overview over methods for using PGRs in plant breeding

Identification (out of 6.1 million accessions)	→	Phenotypic and molecular-genetic characterization, data management
Transfer of superior characteristics		
Introgression	→	Backcrossing of qualitative traits (possibly marker-assisted)
Incorporation ('Basebroadening')	→	Population improvement for quantitative traits (possibly marker-assisted)
'Pre-breeding'	→	Wide crosses
Gene transfer	→	Transformation

5.6.1 Introgression

Introgression aims at improving highly heritable qualitative traits that are governed by one or a few major genes or gene complexes. Traditionally, the classical backcrossing method is used to introgress traits like resistances or restorer genes from wild relatives (= the donor) into breeding materials (= the recurrent parent) (Figure 5.7). The method is particularly effective if the trait to be transferred is dominant. In the case of recessive inheritance, all backcross

FIGURE 5.7
Classical back-crossing for transfer of qualitative traits from a donor into a recipient



progenies need to be selfed in order to identify the carriers of the target allele, before the next backcross of the selected plants can take place.

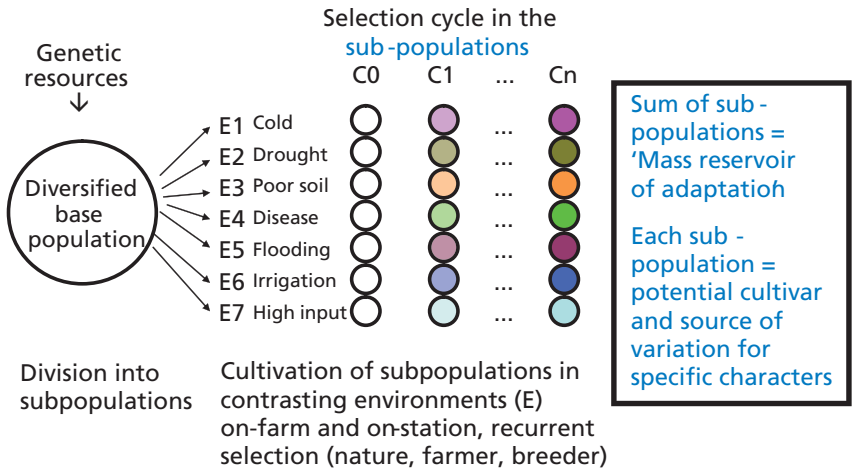
5.6.2 Incorporation

Incorporation, genetic enhancement or base broadening aim to increase the genetic variation for quantitative traits (i.e. traits that are due to many gene loci with small effects) in adapted genetic backgrounds. Various methods of population improvement can be used. The methods will vary depending on the crop species (self- or cross-pollinating) and the available time frame. Initially, selection may concentrate on adaptation traits that are highly heritable; performance traits are selected at a later stage. Diversity and recombination are maximized in the initial phase, with minimal selection intensities. According to the available time frame, two main categories can be distinguished:

- long-term development of synthetic or composite-cross populations and dynamic gene pool management; and
- short-term genetic enhancement to increase the actual variation in breeding populations.

To develop synthetic or composite-cross populations, a large number of accessions of different geographical origin and with maximal genetic diversity are crossed. The resulting population is divided into subpopulations (effective population size N>1000) and the subpopulations are grown for up to 30 generations in a number of different environments. This process is called dynamic gene pool management. At each site, recombination is promoted, and both natural selection and mild mass selection may contribute to adaptation of the individual subpopulations to the site-specific stresses or growing conditions. The sum of all subpopulations has been termed "mass reservoirs of genetic adaptability"

FIGURE 5.8
The concept of dynamic gene pool management



(Simmonds, 1993; Cooper, Spillane and Hodgkin, 2001) and is also understood as a means of *in situ* maintenance of PGR (Figure 5.8). Examples are the barley (*Hordeum vulgare*) composite cross developed at Davis, California, United States of America (Cooper, Spillane and Hodgkin, 2001), dynamic gene pool management in wheat (Goldringer *et al.*, 2001); pearl millet (*Pennisetum glaucum*) composite populations developed in Africa (Niangado, 2001); and the development of locally adapted 'farm cultivars' for ecological agriculture in Europe (Müller, 1989).

In the short term, genetic enhancement of breeding materials, genetic resources are selected for desirable agronomic traits and yield performance, but not for the highest degree of genetic diversity. They are intercrossed, recombined and then selected for adaptation to the target environment. To speed up the process, selected PGR may also be crossed with the breeding materials, and selection for yield traits carried out in the F_2 (50% exotic genome) or BC_1 (25% exotic) generation. The optimal percentage of the exotic genome of the genetic resource (100%, 50% or 25%) in a breeder's population depends on the overall objective; time available and finances; the level of adaptation of the genetic resource; and the yield difference between the genetic resource and the actual breeding population. Direct adaptation of the PGR takes usually longer than selection in F_2 or BC_1 (due to lack of adaptation of the PGR) but will result in materials that are genetically quite different from the actual breeding materials, which can be an advantage. Selection in BC_1 may be preferred over selection in an F_2 population if the PGR is highly unadapted to the target environment. At the same time, selection in the F_2 population is expected to reveal a higher genetic variance, a component of the expected gain from selection (Bridges and Gardner, 1987).

5.6.3 Pre-breeding and wide crosses

Pre-breeding includes basic research to achieve wide crosses, and activities that facilitate the use of exotic materials or wild relatives. It can refer to both qualitative and quantitative traits and the distinction between pre-breeding, introgression and incorporation is not always clear. The main objective is to provide breeders with more 'attractive' genetic resources that are easier to use, such as resistance sources in an acceptable genetic background; or inbreeding-tolerant forms of out-crossing species for hybrid breeding. An example of a very innovative use of wide crosses is the New Rice for Africa (NERICA) developed by the Africa Rice Center (WARDA, www.warda.org). Through crossing the African upland rice, *Oryza glaberrima,* with wetland Asian rice, *O. sativa*, and using embryo rescue and farmer-participatory variety selection, new rice cultivars were developed that combine positive characters (high grain yield and resistances to pests and diseases) of both rice species (www.warda.org/warda1/main/Achievements/nerica.htm).

5.6.4 Gene transfer

Gene transfer is independent of crossing barriers and may therefore increase the usable genetic variation of and beyond the tertiary gene pool. The principal steps for gene transfer from any species into cultivated crops are: gene isolation; gene cloning; gene transfer; and final expression studies in greenhouse and field trials across several generations of progeny. The details of gene transfer go beyond the scope of this chapter. Within the next 10 to 15 years, transformation research hopes to reach the following goals: controlled integration and stable expression of transferred genes; targeted manipulation of multigenic characters; efficient production of transgenes; transgenes, without or with harmless selection markers; and efficient transformation of cell organelles to ensure maternal inheritance, and thereby avoid unwanted horizontal gene transfer (Daniell, Khan and Allison, 2002). Classical examples of the use of gene transfer are the improvement of insect resistance through transfer of *bt* genes from *Bacillus thuringiensis* into crops like tobacco, tomato, maize, rice, cotton and soybean; the improvement of virus resistance through transfer of viral coat proteins in tomato and potato; and the creation of herbicide-resistant crops through transfer of bacterial or fungal genes into sugar beet, tomato and rape. There are also increasing efforts to improve stress tolerance of crops through transfer of genes for improved osmoregulation, heat shock proteins, phytohormone synthesis, and other traits from different organisms into cultivated plants. More information and numerous references on genetic engineering of stress tolerance can be found on the Web site www.plantstress.com.

5.7 UTILITY OF MOLECULAR MARKERS AND GENOME RESEARCH FOR USING GENETIC RESOURCES IN PLANT BREEDING

The utility of molecular markers and genome research in the context of using PGR for crop improvement include:

- diversity studies to distinguish genetically similar or distinct accessions, and to determine individual degrees of heterozygosity and heterogeneity within PGR populations;
- genetic mapping to identify markers in close proximity to genetic factors affecting quantitative trait loci (QTLs), followed by marker-assisted selection (MAS) of desired genotypes in segregating populations;

- exploitation of valuable QTLs from PGR by advanced backcross QTL analysis to combine QTL analysis with the development of superior genotypes or by marker-assisted, controlled introgression of PGR into breeding materials through the development of introgression libraries; and
- association studies to mine directly the allelic diversity of PGR collections and to identify those alleles that are beneficial for important agronomic traits.

5.7.1 Diversity assessment

For an efficient diversity assessment, molecular markers ideally need to be selectively neutral, highly polymorphic, co-dominant, well dispersed throughout the genome, and cost- and labour-efficient (Bretting and Widerlechner, 1995). Genetic markers complying with these requirements are protein markers (i.e. iso-enzymes) and DNA markers such as Restriction Fragment Length Polymorphisms (RFLPs) and Microsatellites or Simple Sequence Repeats (SSRs). Because the development of the latter two marker types requires prior knowledge of DNA sequences, a number of universal, dominant molecular marker types such as Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphisms (AFLPs) have also been employed in PGR diversity studies. However, the latter are not suitable for assessing factors such as mating behaviour or heterozygosity of the germplasm.

Generally, genetic diversity can be measured on three levels: in individual plants, within populations (intrapopulation) and between populations (interpopulation), while populations are considered as groups of randomly interbreeding individuals of one species. The diversity of individual plants is most commonly characterized in terms of the heterozygosity, i.e. the average number of heterozygous gene loci.

At the population level, protein markers and DNA markers are commonly used to calculate, among others, (i) allelic diversity or allelic richness (A; the mean number of alleles per locus); (ii) percentage of polymorphic loci (P; the mean proportion of polymorphic loci); (iii) Nei's average gene diversity (H_e; which denotes the probability that two randomly chosen alleles at a certain locus from a population are different. It is the generalized form of expected heterozygosity assuming Hardy-Weinberg Equilibrium and thus often abbreviated as H_e); and (iv) Shannon's index of diversity (H), which is widely used in ecology but also applied to population genetics (Lowe, Harris and Ashton, 2004).

With the employment of DNA point mutations, such as single nucleotide polymorphisms (SNPs) and small DNA Insertion/Deletions (InDels) as markers for diversity studies, a number of indices have been put forward for variants of a certain DNA sequence in a population. These are (i) the number of polymorphic (segregating) sites (S); (ii) total number of mutations (Eta); (iii) number of haplotypes (h); (iv) haplotype (gene) diversity (H_d); (v) nucleotide diversity (Pi; the average number of nucleotide differences per site between two sequences; Nei, 1987); (vi) nucleotide diversity (Pi (JC); the average number of nucleotide substitutions per site between two sequences (Lynch and Crease, 1990, cited by Rozas *et al.*, 2003); (vii) Watterson estimator Theta (Watterson, 1975); on a base-pair basis it can be interpreted as $4N\mu$ for an autosomal gene of a diploid organism, where N and μ are the effective population size and the mutation rate per nucleotide site per generation, respectively); and (viii) average

number of nucleotide differences (k). It seems noteworthy that indices of nucleotide diversity allow implications that go beyond quantifying the diversity of a population. For instance, the Watterson estimator Theta allows one to infer the effect of selection on a certain locus. However, detailed description of these indices is beyond the scope of this chapter. For further reading refer to Rozas *et al*. (2003).

Diversity between populations is commonly illustrated through graphical presentation of results of multivariate methods (cluster analyses) in the form of dendrograms (e.g. based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) or Neighbour-Joining algorithms) and two- or three-dimensional plots (e.g. Principal Coordinate Analyses). The bases for all cluster analyses are pairwise dissimilarity coefficients (distance/similarity measures) between all respective populations of a study. Some important dissimilarity coefficients for co-dominant marker data are (i) Euclidean Distance; (ii) Modified Rogers' Distance; (iii) Nei's genetic distance; and (iv) Reynolds' dissimilarity (which is based on the co-ancestry coefficient).

Some important similarity coefficients for dominant marker data are (i) Simple matching; (ii) Jaccard (1908, cited by Reif, Melchinger and Frisch, 2005); and (iii) Dice (1945, cited by Reif, Melchinger and Frisch, 2005). A comprehensive account of the dissimilarity indices mentioned here is given by Reif, Melchinger and Frisch (2005) and also by Mohammadi and Prasanna (2003). Considering the partitioning of diversity within and between populations, Wright's Fixation index (F_{ST}), which is calculated from allele frequencies, plays an important role in diversity studies (Lowe, Harris and Ashton, 2004). Besides measuring the partitioning of diversity between and within populations, it can be interpreted as a measure of differentiation between subpopulations, and also as the reduction of heterozygosity of subpopulations due to random genetic drift. In this respect, F_{ST} offers the possibility to calculate gene flow (N_m) between populations according to the formula $N_m = (1 - F_{ST}) / 4F_{ST}$, which can be interpreted as the number of migrants between populations per generation. As the latter indices only apply to co-dominant marker types, Excoffier, Smouse and Quattro (1992) developed a variance-based technique—analysis of molecular variance (AMOVA)—to calculate analogous indices to F_{ST}, which they called Phi_{ST}. AMOVA can also be used to characterize the diversity of populations in terms of variances regardless of the marker type.

It seems noteworthy that comparing data achieved with different molecular marker types, or even measured at different marker loci of the same type, is ambiguous, as diversity measures are relative rather than absolute (Ennos, 1996). For this reason, some authors give diversity indices for a certain marker locus as polymorphism information content (PIC), which provides an estimate of the discriminatory power of a locus (Botstein *et al.*, 1980). The use of PIC values allows the direct comparison of population diversity from different studies, provided that the same marker loci have been used.

A different objective of molecular diversity studies is heterotic grouping of genotypes suitable for hybrid breeding approaches. The principle behind this approach is the search for a correlation between genetic distance and heterosis, i.e. the more distant two genotypes of a crop species are genetically, the more heterozygosity, and therefore heterosis, can

be expected in the hybrid resulting from a cross between them (Melchinger, Coors and Pandey, 1999; Reif *et al*., 2003a, b). Yet, the effect on heterosis and hybrid performance needs to be distinguished, since high heterosis does not necessarily mean high hybrid yield. Recent studies have shown that the correlation between diversity measures and hybrid performance gets stronger when the markers used for diversity assessment are linked to performance QTLs, rather than from using neutral markers (Vuylsteke, 1999; Vuylsteke, Kuiper and Stam, 2000; Jordan *et al.*, 2003).

5.7.2 Genetic mapping and marker-assisted selection

Marker-assisted selection (MAS) can help (i) to select individuals carrying molecular markers that are linked to the trait of interest, instead of performing extensive phenotypic tests (foreground selection); and (ii) to reduce undesired parts of the donor genome, including the linkage drag (background selection). Foreground selection requires a tight linkage between the trait of interest and its flanking markers for which one is selecting. Background selection necessitates genotyping with a larger number of markers, which cover the whole genome.

MAS has proven efficient for the transfer of simply inherited qualitative traits from genetic resources into elite materials using backcrossing procedures. It is particularly useful for traits that are recessive, that can be assessed only after flowering or that are very difficult and expensive to assess. By using a combination of foreground and background selection, the transfer of a monogenic trait from a genetic resource into a breeding line may be completed within three to four generations, instead of the usual six generations of classical backcrossing with the same proportion of the recurrent parent genome (Ragot *et al.*, 1995; Frisch, Bohn and Melchinger, 1999).

MAS for multigenic, quantitative traits at first requires the identification of the genomic regions (QTLs) that affect the trait of interest. In classical QTL mapping, a segregating population (e.g. F_2, F_3 or recombinant inbred population) is developed from two inbred lines. This mapping population is evaluated for the trait(s) of interest. Simultaneously, the population is genotyped with a number of markers and a genetic map is constructed from the marker data. In the final QTL analysis, data is analysed for co-segregation of particular markers with the trait of interest. QTL analysis is then followed by transfer of favourable QTL alleles into elite materials via pure MAS or MAS combined with phenotypic selection.

However, for complex, quantitative traits, the efficiency of QTL mapping and MAS is contested. There are a number of risks that can render MAS inefficient. For example, there may be no selection gain because of: unreliable QTL estimates (too few QTLs, with highly over-estimated effects); QTLs not being expressed in new genetic backgrounds; recombination between marker and QTL; unfavourable alleles of other genes linked to good QTL alleles; or too-high costs for marker analyses. It is therefore essential to use large mapping populations; genotype the mapping population with good genome coverage; assess phenotypic values in multi-environment field trials; cross-validate the gained data; verify QTL effects, using independent population samples, near-isogenic lines or different genetic backgrounds; ensure close linkage between marker and QTL, and verify the linkage by a phenotypic test in all 3 or 4 generations;

increase the marker density around the QTL to allow reduction of the linkage drag; and to optimize individual procedures while taking into account economic parameters. For quantitative traits, where many loci of minor effects are responsible, it is very difficult to obtain reliable, unbiased QTL estimates (e.g. Beavis, 1998; Melchinger, Utz and Schoen, 1998; Utz, Melchinger and Schön, 2000). Prospects for MAS are therefore more promising for traits that are determined by few QTLs with large effects (Melchinger, 1990).

5.7.3 Advanced backcross QTL analysis and introgression libraries

QTL analysis can also be performed in backcross generations derived from crosses of exotic PGR with elite materials. The Advanced Backcross QTL Analysis (AB-QTL; Tanksley and Nelson, 1996) combines QTL analysis with the development of superior genotypes and has been shown to be particularly useful for a trait transfer from poorly adapted germplasm. AB-QTL is therefore of special importance in the use of PGR for crop improvement. The starting point is a segregating generation of a cross between an exotic parent and an elite line that is analysed with as many molecular markers as possible. QTL mapping procedure is delayed until one of the advanced backcross generations ($\geq BC_2$) when lines or testcrosses are evaluated across environments.

To date, the AB-QTL strategy has been applied in several crops, including tomato, rice and barley (Tanksley *et al.,* 1996; Fulton *et al*., 1997, 2000; Bernacchi *et al.,* 1998; Xiao *et al.,* 1996, 1998; Moncada *et al*., 2001; Pillen, Zacharias and Léon, 2003, von Korff *et al*., 2008). Once favourable QTL alleles from an exotic donor are identified, one or two additional backcrossing and selfing generations are needed to derive QTL-bearing near-isogenic lines (QTL-NILs). These carry recurrent parent alleles throughout their genome except for the specific target QTL (Tanksley and Nelson, 1996). QTL-NILs can be used to verify observed QTL effects as well as commercial lines improved for one or more quantitative traits compared with the original recurrent elite line.

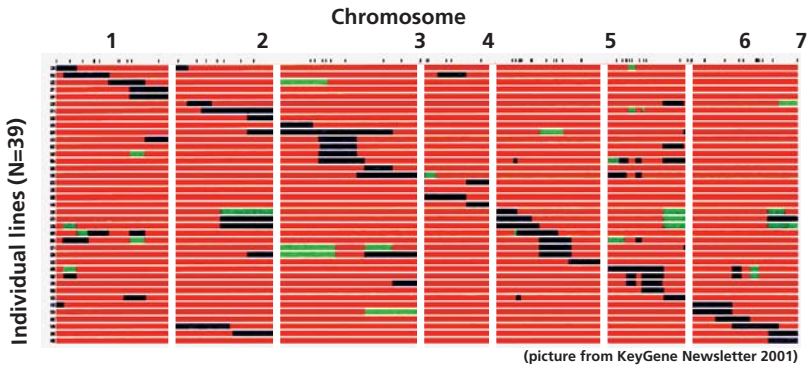
In contrast to the AB-QTL method, Eshed and Zamir (1994, 1995) suggested the approach of establishing a population of NILs such that the donor chromosome segments are evenly distributed over the whole recipient genome. Ideally, the total genome of the exotic donor is comprised in the established set of NILs (Figure 5.9). This NIL population, termed an introgression library, consists of a set of lines, each carrying a single marker-defined donor chromosome segment introgressed from an agriculturally unadapted source into the background of an elite variety (Zamir, 2001).

The procedure of establishing an introgression library implies systematic transfer of donor chromosome segments from a PGR (donor) into an elite line (recurrent parent) by marker-aided backcrossing. Additional self-pollination and marker-based selection lead to NILs homozygous at donor chromosome segments. Such NILs differ from the elite line by only a small, defined chromosomal segment, and phenotypic differences between a line in the library and the nearly isogenic elite line are associated with the single donor chromosome segment (Šimić *et al.*, 2003).

Both introgression library and AB-QTL approaches provide a valuable opportunity to extract quantitative trait alleles for modern crop varieties from exotic PGR. Their main advantage is that the exotic genome is

FIGURE 5.9
Graphical illustration of an introgression library

Using graphical genotypes to illustrate an almost ideal introgression library that covers most of the donor genome



Homozygous region of the donor
Heterozygous regions
Homozygous regions of the recurrent parent

introgressed into the elite line only as small, well defined donor chromosome segments. This reduces unfavourable effects that often impede the use of PGR in practical breeding programmes.

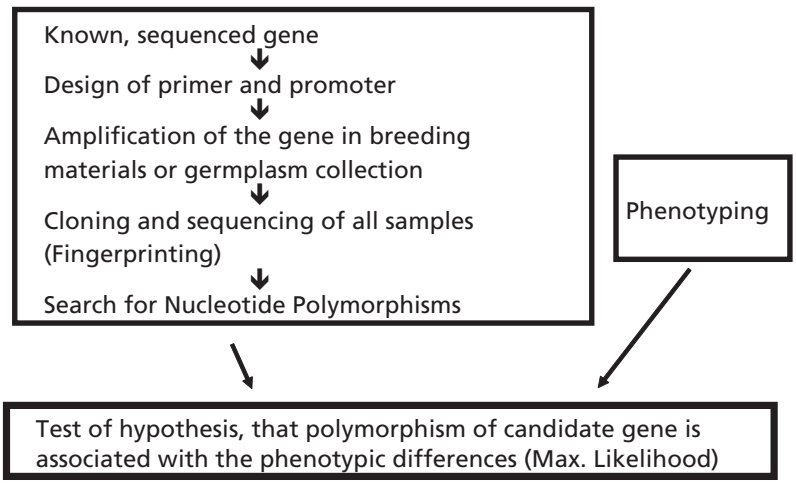
5.7.4 Association studies and direct allele selection

Increased insight into the molecular organization and sequence of plant genomes has led to new methods to mine directly the allelic diversity of PGR. The aim of such studies is to associate sequence polymorphisms within genes or across genomes with phenotypic variants to detect superior alleles affecting agronomically important traits. Such valuable alleles detected within germplasm collections can subsequently be transferred to elite breeding materials via marker-assisted backcrossing using allele-specific markers (direct allele selection; Sorrells and Wilson, 1997) or marker-assisted recurrent selection (D. Hoisington, pers. comm.). The major advantages of association studies over classical QTL mapping experiments is that no segregating population has to be established from two inbred lines, and that the results are not limited to the specific mapping population but can cover the full allelic variation available in natural or breeding populations or gene bank accessions (Jannink, Bink and Jansen, 2001; Jannink and Walsh, 2002).

Associations between DNA sequence polymorphisms and phenotypic trait variation can occur either when the polymorphisms are directly responsible for the functional differences between the alleles of the respective genes, or when the analysed polymorphisms are in linkage disequilibrium (LD) with the functional alleles. LD is defined as a non-random association of alleles at different loci within a population (Falconer and Mackay, 1996).

The basic idea of association mapping can be investigated using two strategies.

FIGURE 5.10
Steps in an association study using the candidate-gene approach



One approach is first to identify candidate genes (i.e. from available databases or gene expression studies) and to re-sequence those candidate genes in plants derived from diverse germplasm accessions (Figure 5.10). The maize gene *dwarf8*, a candidate gene for flowering time and plant height, was used by Thornsberry *et al.* (2001) in a first association study with a crop species. They sequenced *dwarf8* in a representative set of 92 inbred lines and found polymorphisms within the gene to be strongly associated with flowering time. This group of researchers also developed a software suite, TASSEL, (http://www.maizegenetics.net/bioinformatics/index.htm) for analysing LD and for performing association mapping in populations of inbred lines.

A second approach is to analyse a set of randomly chosen molecular markers, evenly distributed across the genome. If such markers are in LD with the genes controlling the trait variation, one will also detect a significant association. The practicability of this approach strongly depends on the level and structure of LD. Low levels of LD would be favourable for high resolution fine mapping within candidate genes, but limit the feasibility of genome-wide association studies. A first attempt to use the genome-wide approach in plants was reported for *Beta vulgaris* subsp. *maritima* using 440 AFLP markers in 106 individual plants from four natural populations (Hansen *et al.*, 2001). Two markers were detected showing significant association with the bolting gene, which is responsible for the vernalization requirement.

Population structure in germplasm collections, which may be unknown to the researcher, can cause spurious associations. Statistical methods were developed by Pritchard, Stephens and Donnelly (2000) and Falush, Stephens and Pritchard (2003) to detect such population structures using a few molecular markers evenly spread across the genome. Removing the effects of population structure increases the power of the association study to detect useful markers.

5.8 THE USE OF GENETIC RESOURCES IN PARTICIPATORY PLANT BREEDING

Genetic resources can be used in a number of ways in participatory plant breeding programmes.

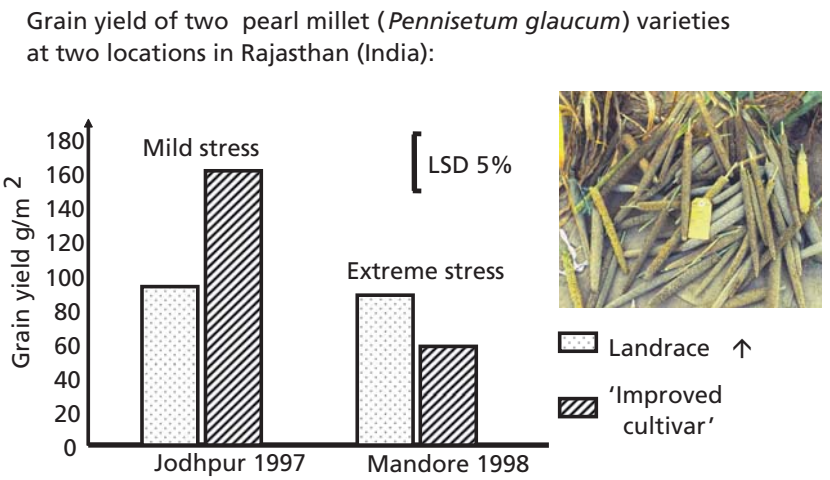
Participatory improvement of diversified populations and potential contribution to in situ *conservation of PGR*

Farmer-participatory improvement of diversified populations combines *in situ* conservation with genetic improvement of PGR to meet farmer's diverse needs as well as the challenges of adaptation to site-specific conditions, climatic variability and climate change. In a first step, farmers may evaluate a range of diverse varieties or germplasm accessions of the target crop and chose accessions that carry traits of interest to them. The diversified base population will then be built through crossing and recombining the farmer-selected materials. Representative seed lots of targeted base populations will be distributed to farmers in contrasting sites with specific selection pressures of a target region (see Figure 5.8 above). Natural and recurrent selection by farmers and breeders will act on the distributed material and lead to the development of new subpopulations that can be excellent sources of variation for specific adaptation and farmer-preferred traits, as well as new trait combinations (via recombination) not previously available. Such a dynamic gene pool approach provides the best opportunity to "offer a wide diversity of material to the wide diversity of farmers" for effective participatory plant breeding (Weltzien *et al.*, 2000).

Use of landraces as genetic resources for specific adaptation to stress environments, climate variability and climate change, and to better serve farmer's and end-user's diverse needs

Breeding for wide adaptation has been found to be inappropriate for extreme stress environments, because of cross-over genotype × environment interactions appearing at low yield levels (e.g. Simmonds, 1991;

FIGURE 5.11
Example of the utility of local landraces as source of adaptation to extreme growing conditions



Ceccarelli *et al.*, 2001; vom Brocke *et al.*, 2002a, b). Cross-over genotype × environment interactions represent the situation where newly bred 'widely adapted' cultivars are inferior to local, indigenous varieties under extreme environmental conditions. An example is given in Figure 5.11. Such interactions may be considered as a hindrance to crop improvement in a target region, but they also offer new opportunities, e.g. selecting and using genotypes that show positive interaction with the location and its prevailing environmental conditions (exploitation of specific adaptation), or genotypes characterized by low frequency of crop failure (Annicchiarico, 2002).

Landraces grown in extreme areas, such as semi-arid to arid regions in Asia and Africa, can represent important PGR in breeding for specific adaptation (Hawtin, Iwanaga and Hodgkin, 1997). They can be donors for individual monogenic traits; sources of new quantitative variation for specific adaptation to stress conditions; and breeding population or crossing partner in the development of improved, locally adapted cultivars for the same or other marginal areas. Strategies for the development of locally adapted germplasm include (Ceccarelli *et al.*, 2001; Witcombe, 2001; Ceccarelli and Grando, 2007):

- decentralization of the breeding process from the international to the national level, and from stations to farmers' fields;
- crossing of elite materials with locally adapted, farmer-preferred cultivars;
- development of different breeding populations for different regions;
- distribution of segregating materials to national programmes; and
- farmer-participatory selection, to increase final acceptance of the improved cultivars.

5.9 OUTLOOK

Numerous methods are available for the use of PGR in crop improvement. The choice mainly depends on the crop, the trait(s) of interest, availability of molecular markers, the chosen time frame, and the finances available. A combination of advanced, molecular techniques with classical and farmer-participatory breeding methods will most probably achieve the desired impact. In order to enhance the utilization of PGR in crop improvement, the Global Plan of Action (FAO, 1996b) proposed a number of measures, among them expanded creation, characterization and evaluation of core collections; increased genetic enhancement and base-broadening efforts; development and commercialization of underutilized species; development of new markets for local varieties and 'diversity-rich' products and concomitant efficient seed production and distribution; comprehensive information systems for PGR; and promoting public awareness of the value of PGR for food and agriculture.

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