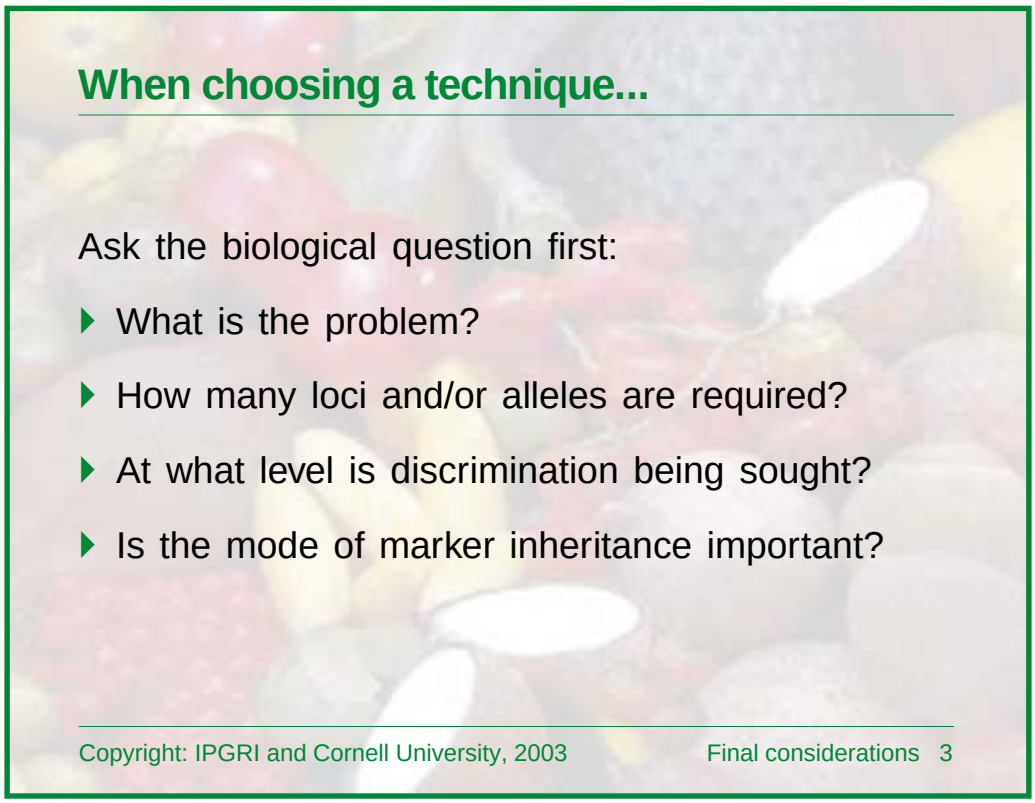
Using molecular marker technology in studies on plant genetic diversity

Final considerations

Contents

- When choosing a technique...
- Practical applications



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What is the problem being addressed?
This is the most important question. The first step is to know exactly what is the biological question one wants to answer with the research. This is essential for choosing the right technique. For instance, for information on population history or phylogenetic relationships, sequence data or restriction site data should be used.

The number of loci and/or alleles required
Will information from a few loci be sufficient or is greater genome coverage required? Allozymes are limited. AFLP detect high numbers of loci. Where hypervariability is required, the best techniques are those based on single-locus, simple-sequence repeats (e.g. SSR).

Discrimination level
At what taxonomic level is the genetic variation being measured: within populations, between species or between genera? Is the selected method appropriate for detecting the desired level of variation?

Mode of inheritance
Should both homozygotes and heterozygotes be identified? Are codominant markers needed (single-locus RFLPs, allozymes, PCR-amplified microsatellites) or will dominant markers suffice (RAPD, AFLP)? If presence versus absence information is sufficient, then any molecular marker technology can be used; but if information about heterozygotes is needed (e.g. population and diversity structure, knowledge on type of inheritance), then only codominant markers such as isozymes or microsatellites should be used.

When choosing a technique... (continued)

Resources:

- Is good quality DNA important?
- Is the right expertise available?
- Well-equipped laboratory
- Costs:
  - Equipment
  - Consumables
- Speed

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DNA availability
RFLP analysis requires large amounts of DNA. Most PCR-based methods require only tiny quantities of easily prepared DNA. In many cases, PCR is performed only to amplify the original amount of target DNA.

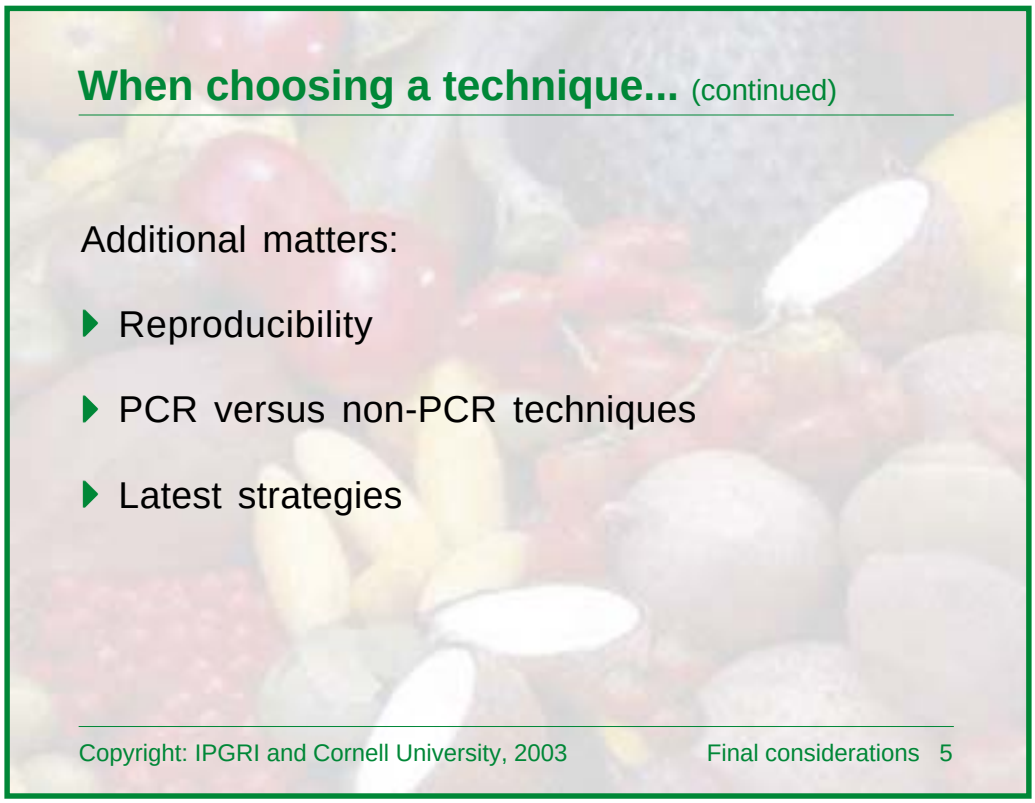
Expertise required
Techniques involving hybridisation or manual sequencing are technically more demanding. RAPDs or SSRs (once the primers are available) are the least demanding.

Availability of laboratory facilities and equipment
Once the biological questions are set, technical and organisational criteria become important in deciding the technology of choice. For example, having (1) access to a suitably equipped laboratory; (2) money to purchase additional equipment and consumables when necessary; (3) a good grasp of many basic laboratory skills; and (4) a basic knowledge of how to set up a experiment.

Costs
In terms of costs, allozymes are the cheapest; RAPD, RFLP and even AFLP are intermediate, with sequencing being still more expensive. The costs of all types of experiments should be considered, because lack of reproducibility of some markers may, in the end, result in higher costs.

For required skills, an extended visit to another laboratory where the relevant techniques are being used often provides invaluable information for setting up the research. Having relevant contacts who may help in those first steps may also prove invaluable.

Speed
How quickly are data needed, and how much time will the equipment allow? PCR-based methods certainly give fast results when primers are available. Hybridisation-based methods are slower. Conventional DNA sequencing is slow, whereas automated sequencing is faster.



Reproducibility
Are robust methods required? For example, will the markers be exchanged? Is more than one laboratory involved? If so, allozymes, RFLPs, SSRs and sequencing are robust, whereas RAPD is not.

PCR versus non-PCR techniques
PCR-based molecular marker techniques open up numerous possibilities and could be considered first, because of their simplicity. Hybridisation-based techniques are more labour intensive, more demanding technically and require different equipment.

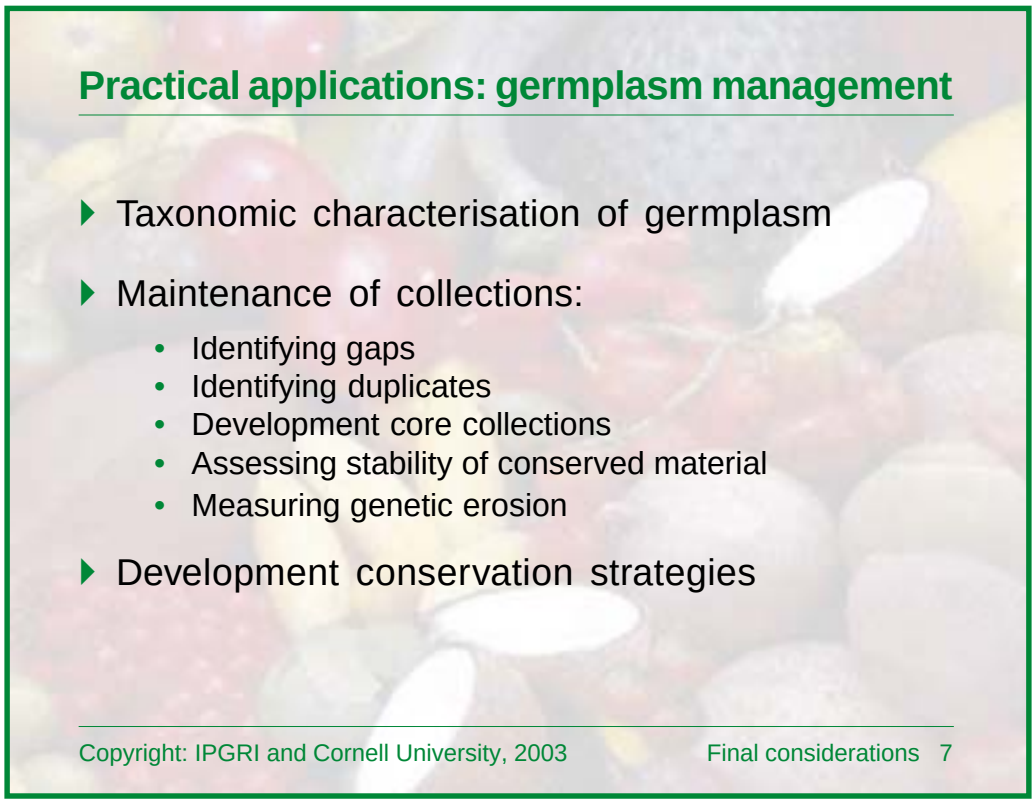
- RAPD is an excellent technique by which to become familiar with PCR. It allows rapid examination of polymorphisms in most, if not all, species of interest, and primers are readily available.
- Other PCR-based markers such as SSR could be applied relatively easily, if primers are already available. More and more, this is the case for many species. Strategies for searching appropriate primers are also improving, and some approaches for searching putative microsatellites rely on sequence databases, circumventing the problem of having to make and screen libraries in the laboratory.
- AFLPs have become a very popular option, although their need for a double PCR and vertical gel electrophoresis makes them more expensive and technically more demanding.

Latest strategies
Costs for sequencing experiments have significantly decreased. Many ESTs are already available for several species. Microarrays, based on either anonymous genomic characterisation or gene expression, are becoming common. Microarray technology is still very demanding, technically and in terms of equipment. Before deciding on it, get acquainted with the techniques, requirements and outputs. A better option might be to consider outsourcing sample analysis and concentrate, instead, on interpreting results and the subsequent decision-making. SNPs are being routinely used in human studies. They are still too expensive for standard applications to genetic diversity studies in plants. Nevertheless, they look at the ultimate level of variation in the DNA sequence the nucleotides, and may well be the future's best molecular marker option when their costs of discovery and application decrease.

Practical applications: genetic diversity studies

- Genetic relatedness and diversity: population genetics
- Studying polymorphism in landraces and cultivars
- Identification of cultivars and taxonomy
- Phylogenetic studies
- Studying domestication and evolution
- Gene flow and introgression
- Comparative mapping

Some of the preceding submodules described real experiments where molecular techniques were applied to answer questions on genetic diversity. Reference lists were also given for the corresponding technology.



Molecular markers may be used in genebank management to:

- Accurately identify germplasm
- Screen germplasm for use by breeders and other researchers conduct routine maintenance of the genebank, which will be streamlined by identifying duplicates, assessing stability through different rounds of regeneration or multiplication and measuring genetic erosion
- Identify gaps in the collection to plan for future conservation and collection activities and for developing core collections where other data will be complemented by ensuring that the allelic richness of a core will be maximised.

Likewise, molecular data can be used to define conservation strategies, both *ex situ* (e.g. collecting strategies) and *in situ.*

Practical applications: germplasm use

- Gene mapping and identification
- Marker-assisted selection in plant breeding
- Detecting somaclonal variation
- Evaluating germplasm for useful genes
- Pedigree analysis
- Hybrid identification

Molecular technologies may help promote germplasm use by providing exact data about the genotypic attributes of plants, including crops. Germplasm characterisation offers information about individual genomic composition and, as such, allows breeders to select promising material based on genotype, as well as on phenotype. The construction of molecular linkage maps have opened up the possibility of locating important agronomic traits in crop genomes and, consequently, of selecting germplasm based on the presence of a particular gene of interest. Introgression of genes from 'donor' germplasm can thus be followed in subsequent generations, using so-called marker-assisted selection, thus facilitating and accelerating traditional selection trials.

Molecular marker technologies are also used to detect somaclonal variation—which may be useful for breeding—that sometimes occurs after regeneration through tissue culture. They can also help in the routine housekeeping activities of a breeding program, such as keeping track of progenies through pedigree analysis, identifying off-types in seed lots and confirming or disproving hybrid purity.

The latest tools for molecular genetics will, hopefully, speed up breeding procedures through such activities as permitting the quick discovery of useful genes in germplasm collections or correlating genotype with phenotype.

In summary

- The most important criteria for choosing a molecular technique for a genetic diversity study are:
  - The biological question driving the study
  - The resources available versus those required
- Applications of molecular technologies cover all the different aspects of genetic diversity analysis, germplasm management and germplasm use

By now you should know

- The criteria that will help you succeed in selecting and applying molecular technologies to the plant genetic resources of interest

Next

Glossary