






Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues

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Foreword

Agriculture is the primary base of livelihood and economy in Bangladesh. It relies mostly on rural populations, which represent 74 per cent of more than a 140 million total, and employs about 55 per cent of the economically actives of the country. Over the last forty years, Bangladesh has been part of the successful story of the Green Revolution, particularly with regard to paddy rice (more than 95 percent of all cereals produced) and wheat production, which both increased, respectively from 14.4 to 39.0 million tonnes, and from 0.03 to 1.90 million tonnes.

However, while the total cereals production was increasing by more than 2.5 times fold, the population was rather tripling, from 53 to 140 million people during the same period. Its technology development kept being confined to rice and wheat, while rain-fed agriculture and several important commodities (pulses, jute, vegetables, fruit, oilseeds and livestock) were left almost unattended. Similarly, technologies for integrated management of soil, water and nutrients, and of pests and diseases geared towards sustainable agriculture were not adequately investigated.

To worsen the whole situation, the productivity gains have been decelerating and the production resources, especially land, water, and genetic resources have been shrinking and degrading. In support of this, the total factor productivity for instance, which moved from -3.2 during the period of 1961-1981 to 1.1 during the one of 1981-2000, remains relatively low. Moreover, the biotic and abiotic stresses, as well as natural disasters have intensified, and inequities and technological divides are widening.

If Bangladesh is to face most of these challenges in the years ahead, agriculture will be required to improve its productivity and produce a more diversified food basket, with greater shares of meat, fish, milk, fruits, and vegetables. It can do so only through further systematic intensification of the use of land, water, and labour; and by properly addressing environmental protection issues, consumer concerns of food safety and quality, and the overall enhancement of rural livelihoods.

To achieve this, biotechnologies, which are endowed with high precision and pace of genetic alchemy of crops, livestock, fish and other aquatic species, forest species and agriculturally important microbes, appear to be a viable option to complement all the efforts actively pursued until now. This explains why the National Agriculture Policy (NAP) has emphasized the role of biotechnology in enhancing and sustaining agricultural production and the will of the Government to take advantage of its potentials. As a matter of fact, the NAP has identified it, along with biodiversity, as a priority area and its promotion as one of its 18 immediate objectives.

As a first move into this option, the Government of Bangladesh, UNDP and FAO embarked on the analysis of the status and prospect of the utilization of biotechnologies, especially modern biotechnology, for sustained and enhanced agricultural production and productivity with the project SPPD BGD/02/005/A/08/12 "**Assessment of Utilization and Potential of Biotechnological Advancement for Agriculture Development in Bangladesh**", completed in 2003. Based on the results of this project, a programme ready to be financed entitled "Biotechnological Advancement for Agricultural Development in Bangladesh " (BAADEB) was formulated by the Government.

This programme aimed to judiciously harness biotechnologies for enhanced agricultural productivity and sustainability towards poverty alleviation and food security. Its long-term vision was to promote biotechnologies for ushering in an evergreen revolution through strengthening national capacity in policy, planning, governance, priority setting, undertaking need-based research and technology development and by establishing research-extension-farmer-consumer-market linkages and public-private-NGO-CSO partnerships. It comprised six interdependent and iterative, yet stand alone, sub-programmes: (i) national policy, strategy and governance, (ii) enabling regulatory measures, (iii) institutional support and strengthening, (iv) biotechnological

interventions for food security and poverty alleviation, (v) human resources development, and (vi) awareness raising and information empowerment.

To ensure the effective implementation of this programme, it was necessary that some special attention be paid to the sub-programme dealing with regulatory measures for two reasons at least. Firstly, specific regulatory measures are inherent to biotechnology development. The "Biotechnology Revolution" most commonly known today as the "Gene Revolution" following concerns that have been constantly raised on some of its products, particularly genetically modified organisms (GMO), has brought along the need for interested countries, to develop preventive measures with regard to environment and public health protection. This need is even more acute in developing countries.

Secondly, without setting up a sound regulatory regime, the country will not fully benefit from biotechnology applications; more specifically, it will not have capacities to absorb imported biotechnologies, and will not be capable of conducting any significant research to develop appropriate biotechnologies suitable to its needs. The technical Cooperation Project TCP/BGD/3102 (D) "Assistance in the formulation of enabling regulatory measures for research and sustainable application of biotechnology" was therefore formulated and launched in May 2008.

Under this TCP, a training course of ten working days for 40 research and technical officers was organized during November 21 – 30, 2008. During this training course the following five modules, namely, agricultural biotechnology; ecological aspects of biosafety; biosafety guidelines including risk analysis; post-release monitoring; post-release monitoring; legal aspects, including plant variety protection of two days each were presented and discussed.

Five experts of the Technical Cooperation among Developing Countries (TCDC) programme were recruited to conduct this workshop as the Resource Persons. They prepared training materials consulting the relevant scientific literature and available training tools and by integrating specific themes adapted to the local environment conditions for the training workshop. During the training course participants were engaged in group exercise for various case studies and presented their outcomes.

After the workshop the TCDC experts submitted detailed individual reports on their respective topics which were reviewed by Lead Technical Officer, National Project Director and National Lead Consultant and recommended for its printing. I would like to congratulate both TCDC experts and National Project Management Unit for their efforts to write and compile the whole document to be printed as a book form. Besides the articles written by the TCDC experts, one article has been included on the Status of Relevant Laws and Regulations on Biotechnology in Bangladesh compiled by Liaquat A. Siddiqui, the Legal Consultant of this TCP/BGD/3102 project.

I hope that this valuable document will be useful for the researchers, extension workers, policymakers, students as a reference book. I once again would like to thank my colleagues at Project Management Unit for taking this initiative to develop this valuable document.

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Chapter 1: Agricultural Biotechnology

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Introduction

Modern biotechnology, including genetic modification and the production of genetically modified organisms, if properly integrated with other technologies, provides powerful tools for the sustainable development of agriculture, fisheries and forestry, as well as meeting the food needs of an expanding and increasingly urbanized population. These tools cover plant improvement to raise and stabilize yields, to improve resistance to pests, diseases and abiotic stresses such as drought and cold and to enhance the nutritional content of foods. Biotechnology is also being used to develop low-cost disease-free planting materials for crops such as cassava, banana and potato and is creating new tools for the diagnosis and treatment of plant and animal diseases and for the characterization and conservation of genetic resources. Animal feeds and feeding practices are also being changed to improve animal nutrition and to reduce environmental waste.

However, with the portfolio of modern biotechnology applications increasing, there is a crucial need to ensure these tools are used judiciously, and that the progress does not overlook potential risks to human health and the environment. This calls for an objective, science-based evaluation system for determining the benefits and risks of each biotechnology application on a case-by-case basis, and for addressing legitimate concerns for the biosafety of each product or process prior to its release. This includes i) evaluating the possible effects on biodiversity, the environment and food safety, ii) weighing the benefits of the product or process against its assessed risks, iii) monitoring the post-release effects of these products and processes to ensure their continued safety. Such an evaluation must take into consideration the experiences of national regulatory authorities in clearing such products.

This module on agricultural biotechnology, targeting regulators and administrators, reviews the very basic scientific concepts and principles employed in producing GMOs, with specific emphasis on the following key areas:

- Basic concepts of biotechnology
- Genes:- structure and formation
- Promoters, vectors and transformation cassettes
- Plant transformation and selection techniques
- Biotechnology for the improvement of animal breeding
- Genetic engineering of microorganisms of interest to agriculture
- Detection methods for Genetically Modified Organisms

Minimal familiarity with these concepts and principles is critical in ensuring pro-active participation to the process of reviewing dossiers and taking part in decision-making.

Introduction to Biotechnology: Basic Concepts and Definitions

Definition of Biotechnology

The term biotechnology was coined in 1919 by Karl Ereky, a Hungarian engineer. At that time, the term meant all the lines of work by which products are produced from raw materials with the aid of living organisms. Ereky envisioned a biochemical age similar to the stone and iron ages. Now biotechnology is broadly defined as the application of scientific and engineering principles to processing of substances by biological agents to provide goods and services. In this definition, the biological agents are mainly microorganisms, animal and plant cells and enzymes. The substances referred to are renewable materials as well as those produced by the biological agents. The goods and services are products of industries concerned with food, beverages, pharmaceuticals and biomedicals. This definition is applicable to both 'traditional or old' and 'new' or 'modern' biotechnology.

Long before the term "biotechnology" was coined for the process of using living organisms to produce improved commodities, people were utilizing living microorganisms to produce valuable products through the fermentation process. A list of early biotechnology applications follows below in Table 1.

Table 1. Traditional biotechnology application.

Providing bread with leaven	Prehistoric period
Fermentation of juices to alcoholic beverages	Prehistoric period
Knowledge of vinegar formation from fermented juices	Prehistoric period
Cultivation of grapes	Before 2000 BC
Manufacture of beer in Babylonia and Egypt	3rd century BC
Wine manufacturing promoted in Roman Empire	3rd century AD
Production of spirits of wine (ethanol)	1150
Vinegar manufacturing industry	14th century
Discovery of the fermentation properties of yeast properties	1818
Description of the lactic acid fermentation by Pasteur	1857
Detection of fermentation enzymes in yeast by Buchner	1897
Discovery of penicillin by Fleming	1928
Discovery of many other antibiotics	≈1945

Since then biotechnology has rapidly progressed and expanded. In the mid-forties, scale-up and commercial production of antibiotics such as penicillin occurred.

The techniques used were:

(a) isolation of an organism producing the chemical of interest using screening /selection procedures, and

(b) improvement of production yields via mutagenesis of the organism or optimization of media and fermentation conditions. This type of "antique" biotechnology is limited to chemicals produced in nature. It is also limited by its trial-and-error approach, and required a lengthy timeframe (years or even decades) for yield improvement.

About two decades ago, biotechnology became much more of a science (rather than an art). Regions of DNA (called genes) were found to contain information that would lead to synthesis of specific proteins. Each of these proteins have their own identity and function, many catalyze (facilitate) chemical reactions, and others are structural components of entities in cells. If one now is able to express a natural gene in simple bacteria such as *Escherichia coli (E. coli)*, a bacterium living in intestines that has become the model organism for much of biotechnology, one can have this bacterium make a lot of the protein coded for by the gene, regardless of its source. The techniques used for this development include:

(a) isolation of the gene coding for a protein of interest,

(b) cloning of this gene into an appropriate production host, and

(c) improving expression by using better promoters, tighter regulation, etc, together these techniques are known as *recombinant DNA techniques.* These will be discussed at some length in the course.

The commercial implications are that a large number of proteins, existing only in tiny quantities in nature, can now be mass-produced if needed. Also, the yields of biochemicals to be produced can be increased much faster than was possible with classical fermentation. As this approach leads to release of genetically altered organisms into the environment, this part of biotechnology is quite strictly regulated at government levels. The main thrust of this entire course is on the development and implementation of such regulatory frameworks at country level.

About a decade ago, "protein engineering" became possible as an offshoot of the recombinant DNA technology. Protein engineering differs from "classical" biotechnology in that it is concerned with producing new (man-made) proteins which have been modified or improved in some way. The techniques involved in protein engineering are more complicated than before, and involve:

(a) various types of mutagenesis (to cause changes in specific locations or regions of a gene to produce a new gene product)

(b) expression of the new gene to form a stable protein

(c) characterization of the structure and function of the protein produced and

(d) selection of new locations or regions to modify as a result of this characterization

Biotechnology applications are driven by a collection of multidisciplinary fields of activities, commonly referred to as *enabling technologies*. Apart from fermentation and genetic engineering/recombinant DNA technology, other important enabling technologies are plant and animal cell culture technology and enzyme technology. The basis of these enabling technologies are the core disciplines of molecular biology, genetics, microbiology, biochemistry, protein chemistry, chemical and process engineering and computer science.

Table 2. An overview of recombinant DNA based biotechnology.

Double helix structure of DNA is first described by Watson and Crick	1953
Cohen and Boyer develop genetic engineering	1973
The first human protein (somatostatin) is produced in a bacterium (E. coli)	1977
The first recombinant protein (human insulin) appears on the market	1982
Polymerase chain reaction (PCR) technique conceived	1983
Lauch of the Human Genome	1990
The first genome sequence of an organism *(Haemophilus influenzae*) is determined	1995
A first draft of the human genome sequence is completed	2000
Over 40 million gene sequences are in GenBank, and genome sequences of hundreds of prokaryotes and dozens of eukaryotes are finished or in draft stage	2005

Overview of Applications of Biotechnology

Since the discovery of recombinant DNA technology, new techniques and applications have been developed that are benefiting mankind in the areas of agriculture, medicine, environment, industry and forensics. The following sections briefly describe some of these applications and their benefits to society.

Industry

Biotechnology is used to develop alternative fuels. Maize starch is converted by yeast into ethanol, which is used to produce gasohol (a gasoline-ethanol mix). Bacteria are also used to decompose sludge and landfill wastes. Through biotechnology, microbes or their enzymes are used to convert biomass into feed stocks, which are used for manufacturing biodegradable plastics (bioplastics) in their tissues. Organisms (microbes and mammals) are used as pharmaceutical factories for producing chemical compounds that are extracted from their products and processed as drugs and other products. Plant and animal fibers are used in making a variety of fabrics, threads, and cordage. Biotechnology is used to improve the quality and quantity of these products. Biopulping is a technique whereby a fungus is used to convert wood chips into pulp for papermaking.

Health/medicine

In the area of health/medicine, biotechnology is used to develop diagnostic tools for identifying heritable diseases. The results of such diagnoses are used in genetic counseling to aid in making informed choices by parents who are predisposed to the birth of children with genetic abnormalities. Diagnostic tools for pregnancy tests, as well as other tests, have also been developed for early detection. Biotechnology is used to produce more effective and efficient vaccines, antibiotics, and other therapeutics. The famous drug penicillin, is a microbial product. Biotechnology is a $30 billion a year industry that has produced some 160 drugs and vaccines. Furthermore, there are more than 370 biotech drug products and vaccines currently in clinical trials targeting more than 200 diseases, including various cancers, Alzheimer's disease, heart disease, diabetes, multiple sclerosis, AIDS and arthritis.

Through the biotechnology of gene therapy, scientists are making attempts at curing genetic diseases by attempting to replace defective genes with healthy ones. A revolutionary strategy is being developed whereby staple foods such as potatoes, bananas, and others are used as delivery vehicles to facilitate the immunization of people in economically depressed regions of the world.

Environment

Developing and using alternative fuels that burn cleaner improves air quality through reduced pollution of the environment. Microbes are used to decompose and clean up contaminated sites by the technology of bioremediation. The use of disease resistant cultivars makes crop production less environmentally intrusive by reducing the use of agrochemicals.

Forensics

Because of the uniqueness or individuality of any individual, the DNA profile or characteristic pattern of nucleotide distribution is used as a powerful basis of identification of individuals in a population. DNA evidence is used in cases involving paternity disputes and family relationships. The application is used in health care and judiciary systems. In health care it is used to diagnose hereditary diseases to predict the chance of an individual inheriting a disease from an affected parent. It can also be used to detect the predisposition of an individual to a cancer, or chromosomal aberrations. In the judicial system, forensic experts use DNA profiling to identify suspects in criminal cases especially where body fluids and other particles like hair and skin samples can be retrieved. DNA profiling is also used in disputed family relations and immigration cases.

Agriculture

Biotechnology provides a more efficient means of crop and animal improvement. Instead of extensive mixing of genes, as occurs in conventional breeding, biotechnology enables targeted gene transfer to occur. The genome of the recipient individual remains intact, except for the introduced gene, thus accelerating breeding programs. Furthermore, biotechnology

enables gene transfer across natural barriers, breaking down mating barriers and creating a sort of "universal gene pool" or "universal breeding population" accessible to all organisms. Biotechnology is used to improve the yield of crop and animal products and their quality such as flavour and shelf life. In addition to these benefits, biotechnology reduces the need for agrochemicals through disease resistance breeding, thereby reducing environmental pollution from chemical runoff. Increased yields and higher food quality reduce world hunger and malnutrition. Molecular techniques are being used to monitor breeding populations and to diagnose animals and plants infected with diseases. Micropropagation techniques are being used widely to generate clonal materials. Rapid large scale clonal propagation of many plant species including trees is feasible through biotechonology.

Genes, Structure and Function

Genes and Heredity

The study of genes and heredity is called genetics. Heredity phenomena have been interesting to humans since before the dawn of civilization. Ancient people were improving plant crops and domesticated animals by selecting desirable individuals for breeding. Genetics as a set of principles and analytical procedures did not begin until 1860s when an Augustinian monk Gregor Mendel performed a set of experiments that pointed to the existence of biological "factors" responsible for transmitting traits from generation to generation. These factors were latter called genes following the discovery of chromosomes and subsequently linkage in the early twentieth century. Up to this point genetics looked at genes as abstract entities that somehow control hereditary traits. Through pure genetic analysis the inheritance of different genes was studied but the physical nature of the gene remained unknown. Further work revealed that chromosomes consist of DNA (deoxyribonucleic acid) and protein, consequently it was found that DNA was the hereditary material.

DNA was thought to be a simple molecule thus many scientists did not believe that it indeed carried/stored the information about an organism's features. How could such information be passes on from one generation to the next? Clearly, the genetic material must have both the ability to encode specific information and the capacity to duplicate that information precisely. What kind of structure could allow such complex functions in so simple a molecule?

The structure of DNA

Although the DNA structure was not known, its basic building blocks had been known for many years. It had been shown that DNA is composed of four basic molecules called nucleotides, which are identical except that each contains a different nitrogen base. Each nucleotide contains phosphate, sugar (of the deoxyribose type), and one of the four bases. The four bases are adenine, guanine (purines) and cytosine and thymine (pyrimidines).

Watson and Crink in 1953 were the first to succeed in putting the building blocks together and finding a reasonable DNA structure. They used DNA x-ray diffraction patterns produced by Rosalind Franklin and Maurice Wilkins and data from Erwin Chargaff. The X-ray data showed DNA molecule to be very long, thin and helical (spiral like) in shape. Chargaff had established certain empirical rules about the amounts of each component of DNA:

1. The total amount of pyrimidine nucleotides (T + C) always equals the total number of purine nucleotides (A + G).
2. The amount of T always equals the amount of A, and the amount of C always equals the amount of G. But the amount of A + T is not necessarily equal to the amount of G + C.

The structure that Watson and Crink derived from these clues is a double helix. Each helix is a chain of nuclotieds held together by phosphodiester bonds, in which a phosphate group forms a bridge between -OH groups on two adjacent sugar residues. The two chains (helices) are held together by hydrogen bonds. Each base pair consists of one purine and one pyrimidine base paired according to the following rule: G pairs with C, and A pairs T (*Refer to diagrams in the power point presentations*).

Elucidation of the structure of DNA caused a lot of excitement in genetics for two basic reasons. First, the structure suggests an obvious way in which the molecule can be duplicated, or replicated since each base can specify its complementary base by hydrogen bonding. Second, the structure suggests that perhaps the sequence of nucleotide pairs in DNA is dictating the sequence of amino acids in the protein encoded by that gene. In other words, some sort of genetic code may write information in DNA as a sequence of nucleotide pairs and then translate it into a different language of amino acid sequences in protein.

The flow of Genetic Information: The Central Dogma

In the early 1950's, Francis Crink suggested that there was a unidirectional flow of genetic information from DNA through RNA to protein, i.e. 'DNA makes RNA makes protein'. This became known as the central dogma of genetics, since it was proposed without much evidence for individual steps. Now these steps are known, DNA is transcribed to an RNA molecule (messenger RNA), that contains the same sequence information, then that message is translated into a protein sequence according to the genetic code.

The genetic Code

The genetic code is the correspondence between the sequence of the four bases in nucleic acids and the sequence of the 20 amino acids in proteins. In has been shown that the code is a triplet code, where three nucleotides (codon) encode one amino acid. Since there are only 20 amino acids to be specified and potentially 64 different triplets (4^3 =64), most amino acids are specified by more than one triplet and the genetic code is said to be degenerate, or to have redundancy. The genetic code has colinearity, this means that the order of the bases in the DNA corresponds directly to the order of amino acids in the protein. Clearly, if the genetic

code is going to be read like we read a sentence of a book, we need to know where to start and stop. As a start signal all proteins start with the amino acid methionine specified by the codon AUG. However, methionine is found in proteins in other places, not just the beginning. Therefore the translational machinery has to find the correct methionine to start and not just any in the sequence, thus the sequences surrounding the initiation AUG codon is important in the translation process. The end of the translated region is determined by one of three codons which basically encode 'stop'. These are UAA, UAG and UGA. If mutations take place in the DNA which create one of the stop codons instead of an amino acid encoding codon, the results may be catastrophic as the resultant protein will shorter than intended. Such proteins would be referred to as being truncated, and very likely to be non-functional. The region between the start methionine and the first stop codon is referred to as the open reading frame (ORF). Finally, the genetic code is virtually universal. Genes taken from plants can be decoded by animal cells, while genes from prokaryotes can be decoded by eukaryotic systems. Without such a universal nature to the code, genetic manipulation and genetic engineering would be much more difficult than it is.

The Gene defined

Historically, a gene is a heritable unit of phenotypic variation, but from a molecular standpoint, a gene is the linear collection of DNA sequences required to produce a functional RNA molecule, or a single transcriptional unit. Genes can be conveniently assigned to one of two broad functional categories: structural genes and regulatory genes. It is the role of the end product of these genes that distinguishes structural and regulatory genes.

1. *Structural genes* code for polypeptides or RNAs needed for the normal metabolic activities of the cell e.g. enzymes, structural proteins, and receptors.
2. *Regulatory genes* code for polypeptides that form proteins whose function is to control the expression of structural genes. With regard to makeup, these genes are like structural genes.

A gene usually occupies a particular location within the chromosome. This location is defined by specific sequences for the start and termination of its transcription. The gene has a specific effect on the organism's morphology or physiology, can be mutated (i.e. changed), and can recombine with other genes. It is a store of information (in the form of nucleotide base sequence), it does not initiate any action but is acted upon. The complete set of genes of an organism, what is called the genetic constitution of the organism, is its genotype. The physical manifestation or expression of the genotype is the phenotype (i.e. the cell's morphology and physiology). If a particular characteristic, such as brown eye color, is a part of an organism's phenotype that is, if it is expressed, it can be said that the individual has the gene for that characteristic. If, however, a particular characteristic is not expressed, one cannot conclude that the gene is absent because gene expression can be repressed.

Genes may be located on either strand of the double stranded DNA. But, regardless of which strand contains a particular gene, all genes are read in a 5' to 3' direction, and the strand containing the particular gene is referred to as the sense or coding strand.

The arrangement of Genes

In eukaryotic organisms each cell contains more than one DNA molecule packaged into individual chromosomes. Along the length of each DNA molecule/chromosome will be found thousands of genes, and although the spacing of the genes is usually apparently random. In bacteria, it is not unusual to have the need to express several genes that are not the same but are related, in that the proteins which are encoded by the genes are required along a common metabolic pathway. Therefore, as all the gene products are needed more or less simultaneously by the cell, it makes sense for the cell to have all such genes together and have a mechanism to express them together. These clusters of genes are known as operons. The most studied operon is the lactose operon in *E coli.* This operon contains three genes which are contagious on the DNA and are required for the utilization of lactose as a metabolic fuel in the cell. The operon also contains all the control sequences (repressor, promoter and operator) needed to ensure efficient expression of the genes together.

Operons do not occur in higher organisms but some similar genes are found in clusters. Many genes in eukaryotes have a distinctive structural feature. More specifically, the nucleotide sequences contain one or more intervening segments of DNA that do not code for the amino acid sequence of the protein product. These non-translated interrupt the otherwise co-linear relationship between the nucleotide sequence of the gene and the amino acid sequences of the protein it encodes. Such non-translated DNA segments in genes are called introns. The pieces that code for mature mRNA are referred to as exons. During transcriptions, the exons are spliced together from a larger precursor RNA that contains, in addition to exons, interspersed introns. The number of exons coding for a single mRNA molecule depends on the gene and the organism but can be a few as one or as many as 50 or more. The origin of intron/exon structure is thought to be extremely ancient and to predate the divergence of eukaryotes and prokaryotes. However, prokaryotes and small eukaryotes (e.g. yeast) have lost their introns during evolution, perhaps because of the strong selective pressure in the organisms to retain a small genome size.

In addition to the introns and exons, the structural features of the eukaryotic gene include regulatory elements, a promoter region, a transcription start site and a transcription termination site (Fig 2.1). Specific cell proteins in the nucleus can bind to the regulatory element sequences of the gene thus controlling the expression of the gene. The promoter region is the sequence of the gene where the specific transcription machinery (assembly of proteins required for transcription) binds to the DNA in order to start transcription. The start site indicates to the transcription machinery where to start and the termination site indicates were to stop transcription of the gene.

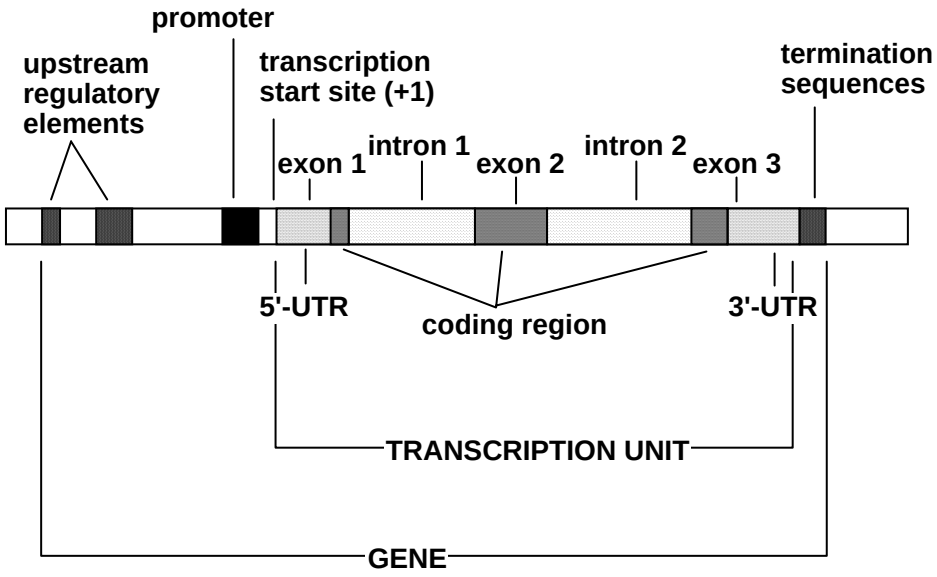

Fig. 2.1. General structural arrangement of the different components making up a eukaryotic gene

Parts of a Gene that are not Transcribed

Promoters

The promoter region of a gene is usually several hundred nucleotides long and immediately upstream from the transcription initiation site and it binds RNA polymerase. There are different types of promoters for different RNA polymerase. Promoters for RNA polymerase II, the polymerase that transcribes genes into mRNA, often contain a consensus sequence 5'-TATA-3', 30 to 50 bp upstream of the site at which transcription begins. Many eukaryotic promoters also have a CAAT box with a GGNCAATCT consensus sequence centered about -75 upstream of the initiation start site. These sequences bind a series of general transcription factors, relatively abundant proteins used to initiate the transcription of nearly all mRNAs. The general transcription factors then facilitate the binding and activation of RNA polymerase II into an activated transcription complex.

Enhancers

Enhancers were described originally as cis-acting sequences that increase transcriptional initiation but, unlike promoters, were not dependent on their orientation or the distance from the transcription start site. It is now apparent that enhancers are generally short (less than 20 to 30bp) sequences that bind specific transcription factors, which then facilitate the assembly of an activated transcriptional complex at the promoter. Most enhancers function whether on the coding or non-coding strand of the DNA (i.e. in either orientation), can act up to several thousand base pairs distant from their promoter target, and are a more general form of cis-

acting regulatory element. Most enhancers are active only in specific cell types and therefore play a central role in regulating tissue specificity of gene expression. Some regulatory elements bind transcription factors that act to reduce transcriptional initiation, and many genes contain a combination of both positive and negative upstream regulatory elements, which then act in concert on a single promoter. This allows gene expression to be controlled very precisely with regard to cell type, developmental stage and environmental conditions. Mutations of gene promoters or enhancers can alter the pattern of expression but not the structure of a particular gene product.

Operators

Operators are nucleotide sequences that lie between the promoter and the structural gene. They are the region of DNA to which repressor proteins bind and thereby prevent transcription. Repressor proteins have a very high affinity for operator sequences. Repression of transcription is accomplished by the repressor protein's attaching to the operator sequence downstream of the promoter sequence (the point of attachment of the RNA polymerase). The enzyme must pass the operator sequence to reach the structural gene's start site. The repressor protein bound to the operator physically prevents this passage, and, as a result, transcription by the polymerase cannot occur.

Attenuators

The attenuator sequences are found in bacterial gene clusters that code for enzymes involved in amino acid biosynthesis. Attenuators are located within so-called leader sequences, a unit of about 162 nucleotide pairs situated between the promoter-operator region and that first structural gene start site of the cluster. Attenuation has a 10 fold effect on transcription. As the level of an amino acid in the cell rises and falls, attenuation adjusts the level of transcription to accommodate the changing levels of the amino acid. High concentrations of the amino acid result in low levels of transcription of the structural genes, and low concentrations of the amino acid result in high levels of transcription. Attenuation proceeds independently of repression, the two phenomena are not dependent on each other. Attenuation results in the premature termination of transcription of the structural genes.

Parts of a Gene that are Transcribed but not Translated

Introns and Splice Junctions

In eukaryotic pre-mRNA processing, intervening sequences (introns) that interrupt the coding regions are removed (spliced out), and the two flanking exons are joined. This splicing reaction occurs in the nucleus and requires the intron to have a 5' –GU, an AG-3' and a branch point sequence. In a two step reaction, the intron is removed as a tailed circular molecule, or lariat, and is degraded. This splicing is directed by RNA-protein complexes known as snRNPs (small nuclear ribunucleoproteins). The snRNPs bind to the conserved sequences to form a spliceosome in which the cleavage and ligation reactions take place.

5' Untranslated Sequences

During the processing of precursor RNA in the nucleus, 3' termini as well as introns are removed. However, precursor RNA always begins with an exon, so that the initial sequences in mRNA are also the first to be synthesized in the precursor RNA. Shortly after initiation of mRNA transcription, a methylguanylate residue is added to the 5' end of the primary transcript. This 5' "cap" is a characteristic of every mRNA molecule, and the transcriptional start or initiation site is also referred to as the capping site. The 5' untranslated region extends from the capping site to the beginning of protein coding sequence and can be up to several hundred base pairs in length. The 5' untranslated regions of most mRNAs contain a consensus sequence of 5' –CGAGCCAUC-3 involved in the intiation of protein synthesis. In addition, some 5' untranslated regions contain "upstream AUGs" that may affect the initiation of protein synthesis and thus could serve to control expression of selected genes at the translational level.

3' Untranslated Sequences and Transcriptional Termination

The 3' end of a mature mRNA molecule is created by cleavage of the primary precursor RNA and the addition of a several hundred nucleotide polyadenylic acid tail. The site for cleavage is marked by the sequence 5' AAUAAA 3' some 15 to 20 nucleotides upstream and by additional uncharacterized sequences 10 to 30 nucleotides downstream. The region from the last protein codon to the polyA addition site may contain up to several hundred nucleotides of a 3' untranslated region, which includes signals that affect mRNA processing and stability. Many mRNAs that are known to have a short half life contain a 50 nucleotide AU rich sequence in the 3' untranslated region. Removal or alteration of this sequence prolongs the half life of mRNA, suggesting that the presence of AU rich sequences in the 3' untranslated region may be a general feature of genes that rapidly alter the level of their expression.

Gene Expression

Genes function through a process called gene expression, a process by which heritable information from a gene is made into a functional gene product, such as protein or RNA. Genes are expressed by being first transcribed into RNA, and may then be translated into protein

RNA

The ribonucleic acid (RNA) is also important in the flow of genetic information. Some viruses use RNA as genetic information. Other organisms that use DNA as the genetic material must first transcribe their genetic information into RNA for the information to be accessible or functional.

RNA is quite similar in structure to DNA. It is a long linear molecule (polymer) that is made up of a limited number of repeating monomers (nucleotides). Each nucleotide is

composed of a sugar, a phosphate, and a base. The sugar is ribose instead of deoxyribose as seen with DNA, hence its name. Unlike DNA, RNA molecules are usually single stranded. RNA molecules have the same bases as DNA except that the pyrimidine base thymine (T) is replaced by uracil (U).

The cell contains three kinds of RNA, namely Messenger RNA (mRNA), Transfer RNA (tRNA) and Ribosomal RNA (rRNA), these correspond to the three basic roles RNA plays in the cell. Firstly, it serves as the intermediate in the flow of information from DNA to protein. The DNA is transcribed (copied) into messenger RNA (mRNA) via an enzyme (RNA polymerase II) and then the mRNA is translated into the protein. Secondly, tRNA molecules serve as adaptors that translate the information in the nucleic acid sequence of mRNA into information designating the sequence of constituents (amino acids) that make up the protein. Finally, the rRNA molecules are important functional components for the molecular machinery (ribosomes) that carries out the translation process.

Transcription and Translation

In all organisms, there are two major steps separating a protein-coding gene from its protein first, the DNA on which the gene resides must be transcribed from DNA to messenger RNA (mRNA), and second, it must be translated from mRNA to protein. RNA-coding genes must still go through the first step, but are not translated into protein.

The first step in gene expression is the ***transcription***, namely the production of a single stranded RNA molecule known as messenger RNA. The nucleotide sequence of the mRNA is complementary to the DNA from which it was transcribed. In other words, the genetic messages encoded in DNA are copied precisely into RNA. The DNA strand whose sequence matches that of the RNA is known as the *coding strand* and the strand from which the RNA was synthesized is the *template strand.*

Transcription is performed by an enzyme called an RNA polymerase, which reads the template strand in the 3’ to 5’ direction and synthesizes the RNA from 5’ to 3’. To initiate transcription, the polymerase first recognizes and binds a promoter region of the gene. Thus a major mechanism of gene regulation is the blocking or sequestering of the promoter region, either by tight binding by repressor molecules that physically block the polymerase, or by organizing the DNA so that the promoter region is not accessible.

In eukaryotes, transcription occurs in the nucleus, where the cell’s DNA is sequestered, the RNA molecule produced by the polymerase is known as the primary transcript and must undergo post transcriptional modification before being exported to the cytoplasm for translation. The splicing of introns present within the transcribed region is a modification unique to eukaryotes. This is a major form of regulation in eukaryotic cells.

Translation is a process by which a mature mRNA molecule is used as a template for synthesizing a new protein. Translation is carried out by ribosomes, large complexes of RNA

and protein responsible for carrying out the chemical reactions to add new aminoacids to a growing polypeptide chain by the formation of peptide bonds.

The genetic code is read three nucleotides at a time, in units called codons, via interactions with specialized RNA molecules called transfer RNA (tRNA). Each tRNA has three unpaired bases known as the anticodon that are complementary to the codon it reads, the tRNA is also covlently attached to the amino acids specified by the complementary codon. When the tRNA binds to its complementary codon in an mRNA strand, the ribosome ligates its amino acid cargo to the new polypeptide chain, which is synthesized from amino terminus to carboxyl terminus. During and after its synthesis, the new protein must fold to its active three-dimensional structure before it can carry out its cellular function.

Regulation of gene expression

Regulation of gene expression refers to the process that cells use to turn the information on genes into gene products. Although a functional gene product may be RNA or a protein, the majority of known mechanisms regulate protein coding genes. Any step of the gene's expression may be modulated, from DNA-RNA transcription modification of a protein.

Vectors, Promoters and Transformation Cassettes

Recombinant DNA Technology : An Overview

Following the elucidation of the structure of DNA and the genetic code, it became clear that many biological secrets were locked up in the sequence of bases in DNA. Technical discoveries in the 1970s led to a new era of DNA analysis and manipulation. Key among these was the discovery of two types of enzymes that made DNA cloning possible. One type called *restriction enzymes,* cut the DNA from any organism at specific sequences of a few nucleotides, generating a reproducible set of fragments. The other type called *DNA ligases,* can covalently join sequences at the termini of restriction fragments. Thus ligases can insert DNA restriction fragments into replicating DNA molecules such as plasmids producing recombinant DNA. The recombinant DNA molecules then can be introduced into appropriate cells, most often bacterial cells, all the descendants from such a single cell, called a clone, carry the same recombinant DNA molecule (Fig 3.1). Once a clone of cells bearing a desired segment of DNA is isolated, unlimited quantities of this DNA can be prepared.

Vectors

A vector is a DNA molecule which can replicate in a suitable host organism, and into which a fragment of DNA may be introduced. Most vectors used in molecular biology are based on bacterial plasmids and bacteriophages. Vectors may need to have the following characteristics:

1. Possess an origin of replication (**ori**), which renders the vector capable of autonomously replicating independent of the main bacterial chromosome.

2. Have a site or sites which can be cleaved by a restriction enzyme, where the foreign DNA fragment can be introduced.
3. There must be convenient markers for identifying the host cell that contains vector with your DNA of interest. A common selection marker is an antibiotic resistance gene. If the host bacteria cells contain the vector then the bacteria will grow in the presence of that antibiotic. Normally the bacteria could not grow in the presence of antibiotic.

In addition to the above features, the vector should be easily introduced into the host organism where it has to replicate and produce copies of itself and the foreign DNA. Furthermore, they should be easy to isolate from the host cell.

Types of Cloning Vectors

Plasmids

Plasmids are circular, double stranded DNA molecules that are separate from a cell's chromosomal DNA. These extrachromosomal DNAs occur naturally in bacteria and in the nuclei of yeast and some higher eukaryotic cells, existing in a parasitic or symbiotic relationship with their host cell. Most naturally occurring plasmids contain genes that provide some benefit to the host cell, fulfilling the plasmid's portion of a symbiotic relationship. For example some bacterial plasmids encode enzymes that inactivate antibiotics. Therefore, a bacterial cell containing such a plasmid is resistant to the antibiotic, whereas the same type of bacterium lacking the drug-resistant plasmid is killed. Plasmids range in size from a few thousand base pairs to more than 100 kilobases (kb).

The plasmids most commonly used in recombinant DNA technology replicate in *E. coli.* Generally these plasmids have been engineered to optimize their use as vectors in DNA cloning. For instance, to simplify working with plasmids, their length is reduced to only ≈3 kb, which is much less than that of naturally occurring *E. coli* plasmids. Most cloning vectors such as pUC18 (Fig 3.1) have a multiple cloning site (MCS), a short region of DNA containing many restriction sites close together (also called polylinker). This allows many different restriction enzymes to be used. In addition to antibiotic resistance gene most modern plasmid vectors use a system for detecting the presence of a recombinant insert, usually the blue/whiteβ-galactosidase system.

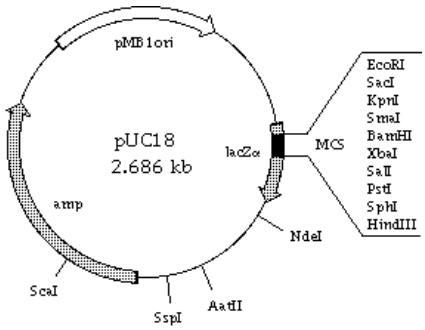

Fig 3.1 pUC18 plasmid cloning vector

Bacteriophage

Bacteriophages,or phages, are viruses which infect bacteria. They can have simple lytic life or more complex lysogenic cycles. One of the best studied phages is bacteriophage λ whose derivatives are commonly used as cloning vectors. The λ phage virion consists of an icosahedral head containing the 48.5kb linear dsDNA geneome, and a long flexible tail. The phage binds to specific receptors on the outer membrane of E. coli and the viral genome is injected through the phage's tail into the cell. The viral genome is linear, its termini are single stranded and complementary. These are called cos ends. The cohesive cos ends rapidly bind to each other once in the cell, produced a nicked circular genome which is repaired by cellular DNA ligase. Much of the central region of the genome is dispensable for lytic infection, and may be replaced by unrelated DNA sequence. There are limits to the size of DNA which can be incorporated into a λ particle, the DNA must be between 75 and 105% of the natural length, i.e. 37 – 52kb. Taking account of the essential regions, DNA fragments of around 20kb (maximum 23kb) can be cloned into λ, which is more than can be conveniently incorporated into a plasmid vector. Another advantage of λ based vectors is that each virion packed with recombinant DNA will infect a single cell. This infection process is about a 1000 times more efficient than transformation with plasmid vectors.

Cosmids

Both λ phage and *E. coli* plasmid vectors are useful for cloning only relatively small DNA fragments. Several other vectors, however have been developed for cloning larger fragments of DNA. One common method for cloning fragments makes use of elements of both plasmid and λ-phage cloning. In this method, called cosmid cloning, recombinant plasmids containing inserted fragments up to 45kb long can be efficiently introduced into*E. Coli* cells. A cosmid vector is produced by inserting the cos sequence from λ-phage DNA into a small *E. coli* plasmid vector about 5kb long. Cosmid vectors contain all the essential components found in plasmids. The cosmid can take foreign DNA inserts that are between 35 and 45kb. Such recombinant molecules can be packaged and used to transform *E. coli.* Since the injected DNA does not encode any λ-phage proteins, no viral particles form in infected cells and no plaques develop on the plate. Rather, the injected DNA circularizes, forming in each host cell a large plasmid containing the cosmid vector and an inserted DNA fragment. Cells containing cosmid molecules can be selected using antibiotic as is done with ordinary plasmid cloning.

A recently developed approach similar to cosmid cloning makes use of larger E. coli viruses such as bacteriophage P1. recombinant plasmids containing DNA fragments up to ≈100 kb can be packaged in vitro with the p1system.

Yeast Artificial Chromosomes (YAC)

YACs are constructed by ligating the components required for replication and segregation of natural yeast chromosomes to very large fragments of target DNA, which may

be more than 1Mb in length. YAC vectors contain two telomeric sequences (TEL), one centromere (CEN), one autonomously replicating sequence (ARS) and genes which act as selectable markers in yeast. YAC selectable markers do not normally confer resistance to toxic substances, as in E. coli plasmids, but instead enable growth of yeast on selective media lacking specific nutrients.

Bacterial Artificial Chromosomes (BAC)

BAC vectors were developed to overcome problems with the use of YACs to clone large genomic DNA fragments. Although YACs can accommodate very large fragments, quite often these fragments turn out to comprise noncontiguous (nonadjacent) segments of the genome and they frequently lose parts of the DNA during propagation (i.e. they are unstable). BACs are generally able to accommodate up to 300 – 350 kb of insert sequence, less than YACs, but they have the advantages not only of stability, but also of the ease of transformation and speed of growth of their E. coli host, and are simpler to purify. The vectors are based on the natural extrachromosomal F factor of E coli, which encoded its own DNA polymerase and is maintained in the cell at a level of one or two copies. A BAC vector incorporates the genes essential for replication and maintenance of the F factor, a selectable marker and a cloning site flanked by a rare-cutting restriction enzyme site and other specific cleavage sites, which serve to enable the clones to be linearized within the vector region, without the possibility of cutting within the very large insert region. BACs are more user friendly than YACs and are now being used extensively in genomic mapping projects.

Promoters

The promoter sequence is the key cis-acting regulatory region that controls the transcription of adjacent coding region(s) into mRNA, which is then directly translated into proteins. Promoters play an important role in the regulation of gene expression at different locations and times during the life cycle of an organism. The study and understanding of the function of their multiple components and the factors associated with their performance have opened up the possibility of modulation of the expression of genes in homologous organisms (i.e. same species) as well as in heterologous organisms (e.g. different species, kingdoms), where foreign promoters together with genes of interest are inserted. As such, promoters have a huge influence in follow-on research and development in biotechnology.

Types of promoters used to regulate gene expression Promoters can be generally divided into:

- ***Constitutive promoters:*** These promoters direct expression in virtually all tissues and are largely if not entirely, independent of environmental and developmental factors. As their expression is normally not conditioned by endogenous factors, constitutive promoters are usually active across species and even across kingdoms.
- ***Tissue-specific promoters:*** These direct the expression of a gene in specific tissue(s) or at certain stages of development. In plants promoter elements that are expressed or

affect the expression of genes in tubers, roots, vascular bundles, other vegetative organs or seeds and other reproductive organs can be found in heterologous systems but it is preferable to work with homologous promoters.

- ***Inducible promoters:*** They are quite popular nowadays because their performance is not conditioned to endogenous factors but external ones that ideally can be artificially controlled. Within this group, there are promoters modulated by abiotic factors such as light, oxygen level, heat, cold and wounding. Since some of these factors are difficult to control outside an experimental setting, promoters that respond to chemical compounds, which are not found naturally in the organism of interest, are of particular interest. Along those lines, promoters that respond to antibiotics, copper, alcohol, steroids and herbicides, among other compounds, have been adapted and refined to allow the induction of gene activity at will and independently of biotic or abiotic factors.

Expression vectors

Cloning a gene encoding a particular protein is only the first of many steps needed to produce a recombinant protein for agricultural, medical or industrial use. The next step is to put gene into a host cell for its expression and the production of protein of interest. For the gene of interest to be expressed in the host cell/organism it must be cloned into a vector that has several distinct sequences/units that provide the different components of a functional gene (see section 2.8 & 2.9). In addition to the characteristics described for cloning vectors, an expression vector must carry a promoter, a polyadenylation and transcription termination sequences within its polylinker. Inserting a coding sequence in proper orientation in between these expression control sequences will result in the expression of the gene in an appropriate host.

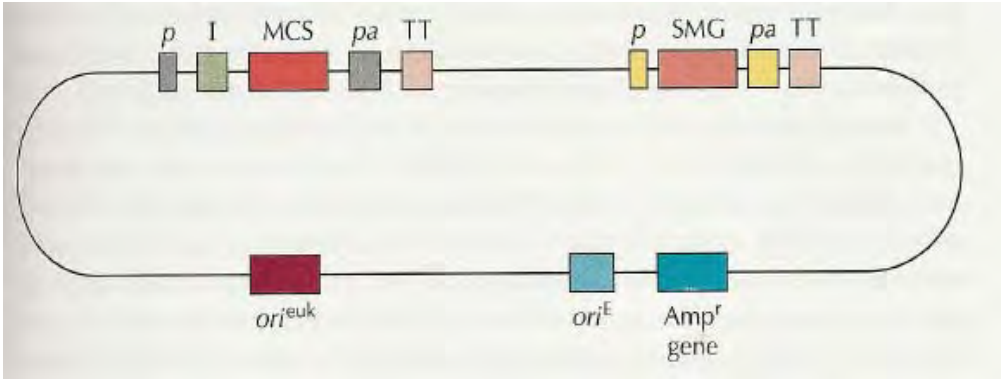

Fig 3.2. Generalised mammalian expression vector. The multiple cloning site (MSC) and selectable gene marker (SMG) are under control of eukaryotic promoter (*p)*, polyadenylation (*pa*), and termination of transcription (TT) sequences. An intron (I) enhances the production of heterologous protein. Propagation of the vector in *E. coli* and mammalian cells depends on the origins of replication ori^{E} and ori^{euk},respectively. The ampicillin gene (Amp^{r}) is used for selecting transformed *E. coli.*

Sometimes it is necessary to fuse some translation control and protein purification elements to the gene of interest (Fig 3.3) or in the expression vector polylinker.

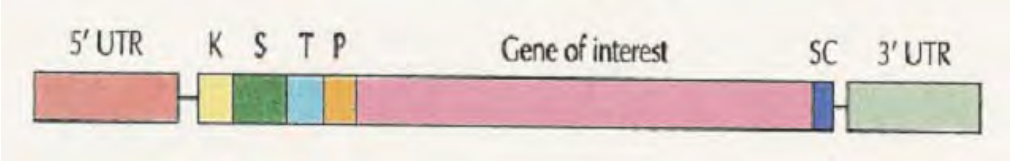

Fig 3.3. A gene of interest fitted with sequences that enhance translation and facilitate both secretion and purification such as a Kozak sequence (K) [5'-ACCAUGG-3', its presence near initiating AUG greatly increases the effectiveness of initiation], signal sequence (S), protein affinity tag (T), proteolytic cleavage site (P), and stop codon (SC). The 5' and 3' UTRs (untranslated regions) increase the efficiency of translation and contribute to mRNA stability.

Plant Transformation and Selection Techniques

Plant transformation

Genetic transformation is the heritable change in a cell or organism brought about by the uptake and establishment of introduced DNA. Transformation encompasses a variety of gene transfer events, characterized by the stability of transformation, the subcellular compartment transformed (nuclear, mitochondrial or plastid) and whether the transferred DNA is integrated into the host genome. Table 4.1 documents the generally accepted definitions of these alternative transformation events.

Table 4.1 Definitions of transformation

Term	Definitions
Stable transformation	Transgene and novel genetic characteristics are maintained during the life of the culture or plant. The transgene is usually, but not necessarily always, integrated into the host genome.
Transient expression	Expression of the transgene is detected in the first few days after its introduction into cells. A subsequent decline in gene activity indicates that expression results from non-integrated DNA.
Integrative transformation	The transgene is covalently integrated into the genome of the host cell. In fertile plants the transgene is inherited by the next generation.
Nuclear transformation	Gene transfere into the nuclear genome of the host cell, as confirmed by cellular fractionation, eukaryotic-type expression or Mendelian inheritance.
Organellar transformation	Gene transfer into the plastid or mitochondrial genome of the host cell, as confirmed by cellular fractionation, prokaryotic-type expression or material inheritance.
Episomal transformation	Viral genomes or 'mini-chromosomes' which replicate independently from the host genome.

Plant Tissue Culture

An important phenomenon and one that is key to plant transformation is that whole plants can be regenerated from single cells. When a plant is wounded mechanically, a patch of soft cells called callus grows over the wound. If a piece of young callus is removed and placed in a liquid culture medium containing the appropriate nutrients and plant growth hormones, the cells continue to grow and divide as a suspension culture. These cells can be planted out onto solid media and will grow to form new calli. The callus will then redifferentiate into shoots and roots, and ultimately a whole plant will be formed. The differentiation of the cells in a callus depends on the relative concentrations of the plant hormones, auxins and cytokinins. If the ratio of auxins to cytokinins is high, then roots develop, shoots develop if the ratio is low. Transformed plants can thus be obtained by introducing DNA into calli or wounded plant tissues such as leaf disks from which callus can be grown and regenerated Fig 4.1.

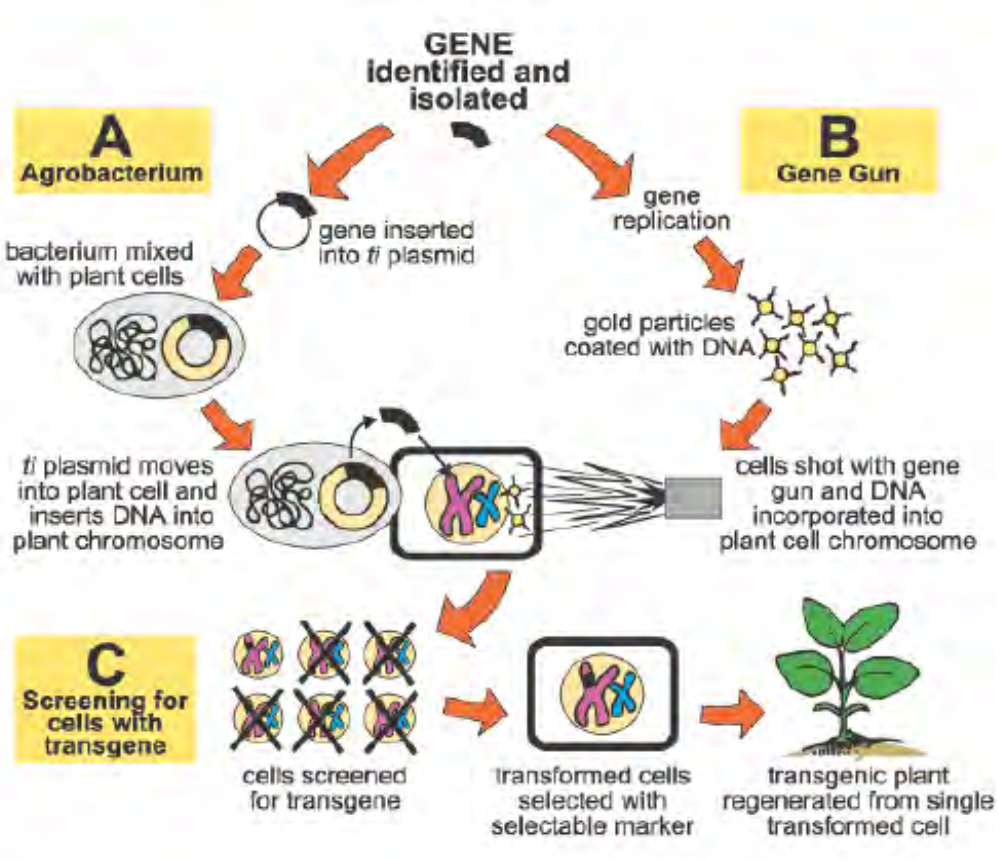

Fig 4.1. Steps involved in the generation of genetically transformed plants using either the *Agrobacterium* of microprojectile bombardment approaches

Plant Transformation Techniques

There is an expanding repertoire of plant transformation approached available, ranging from well proven techniques to highly experimental methodologies. In Table 4.2 these alternative approaches to gene delivery are listed with brief comments on their application, efficiency and limitations. The most widely used techniques are the *Agrobacterium,* microprojectile bombardment ('gene gun' or biolistic) and direct gene transfer to protoplasts. The biolistics technique has been especially useful in transforming monocot species like maize and rice whereas transformation via *Agrobacterium* has been successfully practiced in dicots, but only recently has it been effective in monocots (grasses and their relatives). In general, the *Agrobacterium* method is considered preferable to the gene gun, because of the greater frequency of single-site insertions of the foreign DNA, making it easier to monitor. These techniques are briefly described in the following sections.

Microprojectile bombardment

The technique uses high velocity particles or microprojectiles coated with DNA to deliver exogenous genetic material into the target cell or tissue, which is then cultured *in vitro* and regenerated to produce mature transformed plants.

The particles, either tungsten or gold, are of small size (0.5-5μm) but big enough to have the necessary mass to be accelerated and able to penetrate the cell wall carrying the coated DNA on its surface which once integrated into the cell nucleus can be expressed. Gold particles are chemically inert, although rather costly, and present more uniformity. Tungsten particles, although with some phytotoxicity and of more variable size, are adequate for most studies. Furthermore, the chosen microprojectile should also have good DNA affinity but, at the same time, be able to release it once it has hit the target. DNA coating of surface sterilized particles can be accomplished by binding the DNA, using for instance the calcium chloride method, with the addition of spermidine to protect the DNA. However, recently a report describes the novel use of *Agrobacterium* as coating material for the microprojectiles which are then shot into the target tissue. Once coated the particles are ready for shooting and in some cases macrocarriers are employed to support and accelerate the particles. The macrocarrier is retained by a screen or stopping plate and the particles continue traveling and collide with the target. The DNA, delivered utilizing this direct gene strategy, can be expressed after reaching the nucleus.

Agrobacterium-mediated Plant Transformation

Agrobacterium tumefaciens is a remarkable species of soil-dwelling bacteria that has the ability to infect plant cells with a piece of its DNA. When the bacterial DNA is integrated into a plant chromosome, it effectively hijacks the plant's cellular machinery and uses it to ensure the proliferation of the bacterial population, causing crown gall disease.

The DNA in an A *tumefaciens* cell is contained in the bacterial chromosome as well as in a plasmid known as a Ti (tumor-inducing) plasmid. The Ti plasmid contains a series of *vir*

(virulence) genes that direct the infection process and a stretch of DNA termed T-DNA (~20kb long) that is transferred to the plant cell in the infection process (Fig. 4.2).

Agrobacterium can only infect plant through wounds. When a plant root or stem is wounded it gives off certain chemical signals. In response to those signals, *vir* genes become activated and direct a series of events necessary for the transfer of the T-DNA from the Ti plasmid to the plant cell through the wound. It is not clear how the bacterial DNA moves from the cytoplasm to the nucleus of the plant cell, nor how the T-DNA becomes integrated into the plant chromosome.

Table 4.2. Status of alternative plant transformation techniques.

Gene delivery method	Characteristics
Agrobacterium	Well-established transformation vector for many dicots and a promising vector for gymnosperms. A wide range of oncogenic and disarmed Ti- or Ri-derived plasmid vectors are available. Restricted use with monocots, but valuable for delivery of viral genomes to graminaceous hosts by agroinfection.
Direct gene transfer to protoplasts	Well-established transformation technique with no host range limitation. Plasmalemma permeabilized to DNA by chemical agents or electroporation. Alternatively, genes can be delivered to protoplasts by fusion with DNA in encapsulated liposomes.
Microprojectile Bombardment	A widely authenticated technique for accelerating DNA coated particles into walled cells. No intrinsic host-range limitation. Gene transfer to *in situ* chloroplasts has been achieved.
Microinjection	Effective gene delivery technique offering visual targeting to cell type and intracellular compartment. Labor-intensive to process relatively few cell, and requiring specialist skills and equipment.
Macroinjection of inflorescence	Technically simple approach to deliver DNA to developing floral tissue by hypodermic needle. Germline transformation has not proved reproducible.
Impregnation by whiskers	Suspension cells mixed with DNA and micron-sized whiskers exhibit transient expression and stable transformation.
Laser perforations	Transient expression from cells targeted with a laser microbeam in DNA solution.
Imbition of tissues	Transient and stable expression from tissue bathed in DNA solution of tissues or infiltrated under vacuum.
Floral dip	Stable integration and expression following diping of floral buds in DNA solution.
Pollen tube pathway	Claims of germline transformation by treating pollen or carpels with DNA remain controversial.
Ultrasonication	Stable transformation reported by ultrasonication of explants with DNA. Molecular confirmation is required.

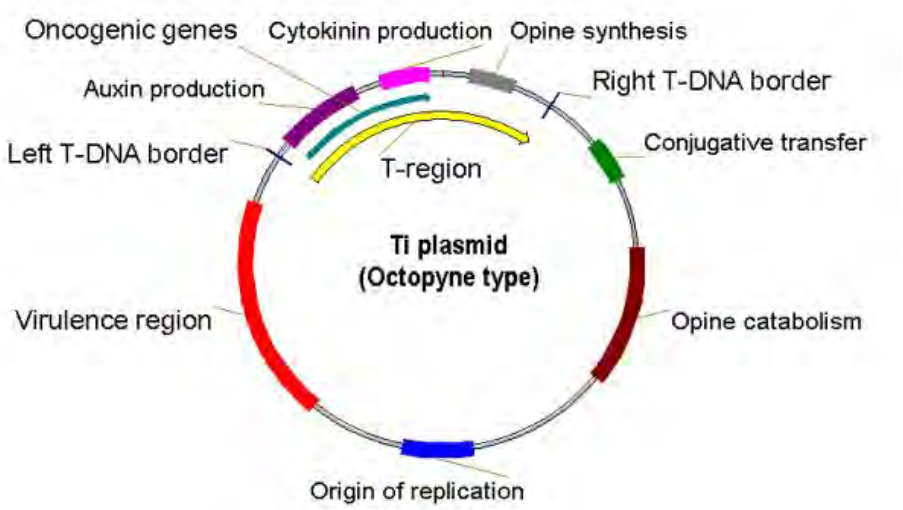

Fig 4.2. Wild type Ti plasmid of *Agrobacterium tumefaciens*

To harness *A tumefaciens* as a transgene vector, scientists have removed the tumor-inducing section of T-DNA, while retaining the T-DNA border regions and the vir genes. The transgene is inserted between the T-DNA border regions, where it is transferred to the plant cell and becomes integrated into the plant's chromosomes. To achieve transformation, *Agrobacterium* cells carrying an appropriately constituted T-DNA based plasmid vector can be inoculated into plants stems, leaf disks etc to facilitate infection and T-DNA transfer to the plant cells. These explants that have been co-cultivated with the *Agrobacterium* can then go through various tissue culture steps leading to the selection and production of transformed cells and plants.

Protoplast Transformation Techniques

Protoplasts are plant cells in which the cell wall has been removed. Therefore protoplasts can behave like animal cells, which have no cell wall barrier. Plant regeneration from single protoplasts is possible due to the totipotency of plant cells. Removal of the cell wall is achieved by treating the plant material (leaves, tissue cultures, suspended cells, etc) with a cocktail of enzymes including pectinases, cellulases, and/or hemicellulases in an appropriate incubation medium of the right osmolality. After removal of the cell wall, the protoplasts must be kept immersed in a solution of the appropriate concentration to prevent them from bursting. Also the protoplasts must be incubated in a culture medium of the correct osmolality until wall formation occurs.

Different approaches exist for the insertion of transgenes into protoplasts through the plasma membrane. These include: chemical, electroporation and microinjection techniques.

Chemical Techniques

The most common methods are polyethylene glycol (PEG), Ca^{2+} -DNA co-precipitation and liposomes. PEG is the most widely used, employing solutions of 10-15% PEG, with high calcium content and high pH. After mixing isolated DNA and protoplasts, followed by different washes, DNA and protoplast fusion takes place. Here PEG alters the plasma membrane properties causing reversible permeabilization enabling exogenous macromolecules to enter the cell cytoplasm.

Ca^{2+} -DNA co-precipitation depends on the formation of a co-precipitate of plasmid DNA and calcium phosphate. On contact with protoplasts under high pH conditions, the co-precipitate trespasses the cell.

Liposomes, these are negatively-charged spheres of lipids, are also employed for DNA transfer. DNA is first encapsulated into the liposomes and these are fused with protoplasts employing PEG as a fusogen.

Electroporation

Electrical pulses are applied to the DNA-protoplast mixture, provoking an aincrease in the protoplast membrane permeability to DNA. It is much simpler than the chemical method, giving attractive results. However, the electrical pulses must be carefully controlled as cell death can occur above a certain threshold. The pulses create the transient formation of micropores in the lipid bilayer which last for a few minutes, allowing for DNA uptake.

Microinjection

This technique was originally designed to transform animal cells, later it gained importance and interest in transforming plant cells. However, in plant cells the existence of a tough cell wall, a natural rigid barrier, as well as the presence of vacuoles which can produce cell death after breakage due to the release of hydrolases and toxic metabolites, and in some instances where vacuoles surround the nucleus make microinjection impossible. Therefore, protoplast rather than intact plant cells are more suitable for microinjection, and thus subsequent genetic modification. Clearly, this method is rather labor intensive and requires specialized microequipment for the manipulation of host protoplasts and DNA. However, some success in transforming both monocotyledonous and dicotyledonous species has been achieved employing this technique.

Selection of Successfully Transformed Tissues

Following the gene insertion process, plant tissues are transferred to a selective medium containing an antibiotic or herbicide, depending on which selectable marker was used in the expression cassette. Selectable markers are those which allow the selection of transformed cells, or tissue explants, by their ability to grow in the presence of the antibiotic or herbicide. Only cell/plants expressing the selectable marker gene will survive and it is assumed that these plants will also possess the transgene of interest. Thus subsequent steps in the process

will only use these surviving plants. In addition to selecting for transformants, such markers can be used to follow the inheritance of a foreign gene in a segregating population of plants.

Most transformation cassettes also include screenable markers/reporter genes that encode gene products whose enzyme activity can be easily assayed, allowing not only the detection of transformants but also and estimation of the levels of foreign gene expression in transgenic tissue. Markers such as β-glucuronidase (GUS) and luciferase allow screening for enzyme activity by histochemical staining or fluorimetric assay of individual cells and can be used to study cell-specifics as well as developmentally regulated gene expression.

Selectable marker genes

The selectable functions on most general transformation vectors are prokaryotic antibiotic resistance enzymes which have been engineered to be expressed constitutively in plant cells. In some experiments, enzymes affording protection against specific herbicides have also been used successfully as dominant marker genes. The selective agent concerned must be able to exert stringent selection pressure on the plant tissue concerned.

Neomycin phosphotransferase (NPT-II) Gene

Neomycin phosphotransferase-II (NPT-II) is a small (25kd) bacterial enzyme which catalyses the *ortho*-phosphorylation of a number of aminoglycoside antibiotics including neomycin and kanamycin. The reaction involves transfer of the γ-phosphate group of ATP to the antibiotic molecule, which detoxifies the antibiotic by preventing its interaction with the target site-the ribosome. This transfer reaction has been exploited to develop a sensitive solid phase assay for the enzyme. The total proteins are first extracted from the tissues to be analyzed for the presence of the transgenes.

Chloramphenicol Acetyltransferase Gene

This is the chloramphenicol resistance (*cat)* gene encodes the enzyme chloramphenicol acetyltransferase (CAT) and was the first bacterial gene to be expressed in plants. The enzyme specifically acetylates chloramphenicol antibiotics to the 1 -, 3 -, and 1,3-acetylated derivatives, which are inactive. Although not used as a selection system in plants, the gene is used frequently as a reporter gene in plant promoter work.

β-glucuronidase Gene

The *E. coli* β-glucoronidase gene has been developed as a reporter gene system for the transformation of plants. β-glucuronidase, encoded by the *uidA* locus, is a hydrolase that catalyses the cleavage of a wide variety of β-glucuronides, many of which are available commercially as spectrophotometric, fluorometric and histochemical substrates. There are several useful features of the GUS gene which make it a superior reporter gene for plant studies. Firstly, many plants assayed to date lack detectable glucuronidase activity, providing a null background in plants. Secondly, glucuronidase is easily, sensitively and cheaply

assayed both *in vitro, in situ* in gels and is robust enough to withstand fixation, enabling histochemical localization in cells and tissue sections. The preferred histochemical substrate for tissue localization of GUS is 5-bromo-4chloro-3-indolyl-β-D-glucuronide (X-gluc). The advantage of these substrates is that the indoxyl group produced upon enzymatic cleavage dimerizes to indigo which is virtually insoluble in an aqueous environment. The histochemical assay for GUS consists of soaking tissue in substrate solution and watching for blue color to appear.

Luciferase Gene

The luciferase (*luc)* gene isolated from *Photinus pyralis* (firefly) encodes the enzyme catalyzing the ATP/oxygen-dependent oxidation of the substrate luciferin which produces emission of light (bioluminescence). As a reporter, the gene is the basis of highly sensitive assays for promoter activity and for protein targeting sequences, involving the measurement of light emission using liquid scintillation counter photomultiplers, luminometers, X-ray film exposure or sensitive camera/film.

Molecular Analysis of Transgenic Plants

Analysis for the transgenes at the molecular level is mainly carried out by Polymerase Chain Reaction(Box 7.1) and genomic Southern analysis. Polymerase chain reaction shows the presence of the transgene where as stable integration of the transgene is confirmed by genomic Southern analysis. To analyze DNA where *Agrobacterium* vectors are used for transformation, it is important to prepare plant DNA from sterile tissue, as contamination with *A.tumefaciens* DNA will interfere with the interpretation of the results. Genomic Southern analysis also yields information on the copy number of the integrated DNA sequences, whether any multiple inserts are tandemly linked or dispersed, and on the stability of this DNA in the F_1 progency of the transformed plants.

Biotechnology in Animal Production

Biotechnology has a number of applications in livestock production. It is being used to hasten animal growth, enhance reproductive capacity, improve animal health and develop new animal products. This chapter looks at these biotechnology applications and how they are impacting on animal improvement and production.

Biotechnology in Animal Breeding

Animal breeding is a field related to a whole range of biotechnologies. The impact of a biotechnology can be measured by the influence it has on genetic progress. According to the type of biotechnology considered, different component of genetic progress may be affected: accuracy of prediction, generation interval, intensity of selection and genetic variance.

The first type of biotechnologies affects the efficiency of male and female reproduction: artificial insemination, multiple ovulation, in-vitro-fertilization, ova pick-up, embryo-transfer,

twining, sexing of semen and embryo cloning and selfing. The impact of these technologies is mainly in the enhanced distribution of superior germplasm and the selection intensity, but also in the accuracy obtained when testing animals.

A second group of biotechnologies can improve determination of the genetic merit of animals. These are all the techniques related to quantitative or economical trait loci (QTL), their detection and use. Their main feature is the early availability in life, therefore allowing an earlier and more accurate selection. Two directions of research exists: detection of markers for the unknown QTL and direct use of a potential candidate genes as QTL.

Animal Reproduction

Artificial insemination (AI)

This is the process of semen collection from a desired bull to be used in fertilizing many cows. The semen can be diluted, and preserved through cryopreservation. The technique can enable a single bull to be used simultaneously in several countries up to 100,000 inseminations a year. The high intensity and accuracy of selection arising from AI can lead to a four-fold increase in the rate of genetic improvement in dairy cattle relative to that from natural mating. Since its widespread use in the 1950s, AI has been a very successful biotechnology, enhancing greatly the genetic progress. Use of AI can reduce transmission of venereal diseases in a population and the need for farmers to maintain their own breeding males, facilitate more accurate recording of pedigree and minimize the cost of introducing improved stock. Though widely used in dairy cattle breeding AI had its greatest impact in broiler chicken production. In the 1950s the chicken was a luxury item costing around $30 at today's prices; Harry S Truman won the 1947 US Presidential election with the slogan "a chicken in every pot".

Embryo transfer (ET)

Although not economically feasible for commercial use on small farms at present, embryo technology can greatly contribute to research and genetic improvement in local breeds. There are two procedures presently available for production of embryos from donor females. One consists of superovulation using a range of hormone implants and treatments, followed by AI and then flushing of the uterus to gather the embryos. The other, called *in vitro* fertilization (IVF) consists of recovery of eggs from the ovaries with the aid of the state-of-the-art ultrasound-guided transvaginal oocyte pick-up (OPU) technique. When heifers reach puberty at 11-12 months of age, their oocytes may be retrieved weekly or even twice a week. These are matured and fertilized in vitro and kept until they are ready for implantation into foster females. In this way, high-value female calves can be used for breeding long before they reach their normal breeding age. IVF facilitates recovery of a large number of embryos from a single female at a reduced cost thus making ET techniques economically feasible on a larger scale. Additionally, IVF makes available embryos suitable for cloning. However, embryo transfer is still not widely used despite its potential benefits.

Embryo sexing

Technologies for rapid and reliable sexing of embryos allow the generation of only the desired sex at specific points in a genetic improvement programme, markedly reducing the number of animals required and enabling increased genetic progress. A number of approaches to the sexing of semen have been attempted, and several have been reported as successful. However, the only method of semen sexing that has shown any promise has been the sorting of spermatozoa according to the DNA content, by means of flow cytometry. Embryo sexing has been attempted by a variety of methods, including cytogenetic analysis, assays for X-linked enzyme activity, analysis of differential development rates, detection of male-specific antigens, and the use of Y-chromosome specific DNA sequences.

Animal cloning

Animal cloning may be produced by embryo splitting and nuclear transfer (somatic cell cloning). These offer the possibility for creating large clone families from selected superior genotypes which, in turn, can be used to produce commercial clone lines. The process of somatic cell cloning involves replacing the DNA in an unfertilized oocyte with DNA from a somatic (body) cell. The oocyte has the ability to reprogram the somatic cell DNA so that the unfertilized oocyte can develop as an embryo and, in some cases, give rise to healthy calves which have DNA that is entirely from the somatic cell. Because it is possible to obtain an unlimited number of genetically identical somatic cells from an animal, cloning is a technology that can be used for producing genetically identical calves. However, the somatic cell can also be genetically manipulated prior to being introduced into the oocyte, so cloning is also a convenient method of making transgenic cattle.

Genetic markers and marker-assisted selection

A genetic marker for a trait is a DNA segment which is associated with, and hence segregates in a predictable pattern as the trait. Genetic markers facilitate the “tagging” of individual genes or small chromosome segments containing genes which influence the trait of interest. Availability of large numbers of such markers has enhanced the likelihood of detection of major genes influencing quantitative traits. The process of selection for a particular trait using genetic markers is called marker assisted selection (MAS). MAS can accelerate the rate of genetic progress by increasing accuracy-of selection and by reducing the generation interval. However, the benefit of MAS is greatest for traits with low heritability and when the marker explains a larger proportion of the genetic variance than does the economic trait.

Marker identification and use should enhance future prospects for breeding for such traits as tolerance or resistance to environmental stresses, including diseases.

Two types of marker can be considered. First, markers that are sufficiently close to the trait gene on the chromosome such that, in most cases, alleles at the marker and the trait gene are inherited together. This type of marker is called a linked marker. At the population level

alleles at linked markers cannot be used to predict the phenotype until the association between alleles at the marker and alleles at the trait-gene is known (called 'phase'). To determine phase, inheritance of the marker and trait gene has to be studied in a family. However, information on phase is only valid within that family and may change in subsequent generations through recombination.

The second type of marker is a functional trait. These markers are called 'direct' markers. Once the functional polymorphism is known it is possible to predict the effect of particular alleles in all animals in a population, without first having to determine the phase. Therefore, 'direct' markers are more useful than 'linked' markers for predicting the phenotypic variation of target traits within a population. A further complication is that the mechanisms of genetic control differ between traits. The variation seen in some traits is directly controlled by a single gene (monogenic traits), which may have a limited number of alleles. In the simplest situation a gene will have two alleles: one allele will be associated with one phenotype and another allele with a different phenotype (e.g. black versus brown coat color in cattle: the brown coat color occurs as a result of mutation in the melanocyte hormone receptor gene, which results in the creation of a different allele that alters its function)

However, the traits that are important in livestock production are generally more complex and have a very large range of variation in the observed phenotype. Growth rate and milk yield are examples of two traits that exhibit a continuous phenotypic variation. Such traits are called quantitative traits. The variation in quantitative traits is controlled by several genetic loci (called quantitative trait loci [QTL]), each of which is responsible for a small amount of the overall variation. The behavior of genes (including major genes) that control a trait is likely to be dependent on the genetic background. The myostatin allele responsible for double muscling in Belgian Blue cattle is also found in other breeds, however, the phenotype associated with the allele is variable between the breeds. This suggests that there are genes at other loci in the genome that act to modify the phenotypic expression of the major gene. Thus, information is required not only on the major genes that control a trait, but also on the interactions between genes. It is therefore premature to start using DNA-based selection widely. However, some DNA tests for specific polymorphisms are being offered commercially, e.g. GeneSTAR tests for tenderness (based on variations in the calpastation gene) and marbling (based on variations in the thyfoglobulin gene), and the Ingenity test for fat deposition (based on variations in the leptin gene). These tests can be used by breeders and evaluated in their populations.

Transgenic Animals

The term transgenic animal refers to an animal in which there has been a deliberate modification of the genome. Foreign DNA is introduced into the animal, using recombinant DNA technology, and then must be transmitted through the germ line so that every cell,

including germ cells, of the animal contain the same modified genetic material. The first transgenic animal was a mouse produced in 1982 by microinjection of DNA into the fertilized single cell oocyte. This ground breaking work was published in Nature and the cover of the magazine showed a comparison of the transgenic mice and their non transgenic litter mates. The transgenic mice were huge, twice the size of their litter mates. This image stimulated the imaginations of both the public and scientists and created a tremendous amount of speculation about the potential impact of transgenic technologies for agricultural animals. It was surmised by inserting a single growth regulating gene into an animal of agricultural value at growth rate and feed efficiency could be greatly increased and fat deposition reduced, transforming the entire meat animal industry. Furthermore, many other applications, including, enhanced milk production , production of milk with novel properties, enhanced disease and parasite resistance and increased wool production were imagined. By 1985, transgenics has been produced in pigs, sheep, and cattle with chicken following a little later. Since then there has been a slow, but relatively steady, effort to apply transgenic technologies to agricultural species. Initially, technical limitations, cost and lack of understanding about genes and their regulation severely limited progress, particularly in species such as the cow.

The first transgenic technology has limitations: less than 1% of embryos injected and 10% of animals born are transgenic, genes can only be added, not replaced or deleted, because multiple copies are inserted at random, correct regulation of gene expression is difficult.

To overcome these problems in the mouse, embryonic stem cells (ES cells) have been developed. These cells can be grown stably in culture for many passages and transformed with gene constructs. The constructs not only permit transformed cells to be selected but also gene targeting to be accomplished. Transformed cells are introduced into the blastocoel cavity of an embryo, produce a mosaic (chimaeric) animal and contribute to the germline. After one generation this will produce a germline transgenic animal. This technique, in principle, produces 100% transgenic animals and, by gene targeting a much wider variation of genetic modifications (such as gene knock-outs). For many years, several labs world wide have tried to produce ES cells in farm animals, although some success has been claimed, no robust and repeatable method has been published. Indeed, ES cells can only be produced even in mice from a limited number of inbred strains.

Recently a robust method for gene targeting in cattle, using somatic cell cloning technology, has been developed. Gene targeting is the insertion of a transgene, or any exogenous DNA sequence, into a specific, targeted site in the host DNA. The technique is more complex than random gene insertion but gene targeting is a much more powerful technology because it can be used to inactivate genes, insert new genes into predetermined sites or replace one variation of a gene with another variation. It overcomes many of the limitations of random gene insertion by microinjection. Because the insertion site is predetermined, a series of transgenic founder animals can be made, including both males and females, which can be mated to make homozygous offspring. An even simple approach to

making homozygous transgenic animals is to sequentially insert a copy of the transgene into one member of a pair of chromosomes and then insert a second copy into the other chromosome without germ line transmission of the transgene.

Some research groups have developed a rejuvenation system for bovine fibroblast cells. The system involves making a genetic modification in a fibroblast cell line established from a bovine fetus. Because the cells only grow for a limited number of cell divisions in culture only one genetic modification can be made before the cells become senescent and stop dividing. The cells are then used in a cloning procedure to produce cloned fetuses. Young healthy cell lines can then be made from the fetuses and used for a second round of genetic modification. When the genetic modifications are complete then the final fetal cell line can be used for making calves.

A second advancement in cattle transgenics, which has been accomplished recently, is microchromosome transfer. A microchromosome is different from a typical transgene in a couple of characteristsic. First, a typical transgene consists of a couple of gene sequences and may be up to 25,000 DNA bases long, whereas, a microchromosome typically consists of millions of DNA bases and can contain either very long genes or potentially hundred of genes. Second, a typical transgene must integrate into the host DNA, either randomly or targeted to a specific sequence, to be carried along through cell division. Microchromosomes do not integrate but replicate on their own and are carried along during cell division as independent chromosomes. Human-derived microchromosome have been successfully inserted into cattle. A microchromosome was needed because there was need to transfer the human antibody genes into cows. Antibody genes are very complex and are up to several million DNA bases long, well beyond the capacity of a typical transgenic vector. The microchromosome is stable in cattle and appears to have no harmful effects on the animals.

Since the production of the first transgenic mice, work in cattle has focused primarily on technology development. At this time, many technical hurdles for application of transgenic technology to cattle have been overcome. In fact, transgenic technologies for the cow are comparable to that of the mouse. Two kinds of applications for transgenic technology in cattle are being pursued. One involves genetic modifications that are aimed at improving the efficiency of food (meat or milk) production. The second is the production of novel products, such as pharmaceutical proteins for human health care.

Transgenic Animals for Food Production

There are a few research reports describing the use of transgenic technologies in cattle directed towards a food production application. Brophy *et al*, (2003) introduced additional copies of bovine beta or kappa casein into dairy cattle and evaluated the effect on milk production and composition. Transgenic offspring had an 8 to 20% increase in beta casein and a two-fold increase in kappa casein. In pigs several attempts have been made at improving growth and composition by the addition of transgenes. In one study expression of

an exogenous insulin-like growth factor gene in the muscle of pigs resulted in significant reduction in fat and an increase in lean muscle in gilts but not boars (Pursel *et al,* 1999). In another study, a widely expressed exogenous growth hormone gene tended to increase live weight gain, improve feed efficiency and reduce back fat thickness (Nottle *et al,* 1999).

Although these studies demonstrate the feasibility of improving food production efficiency with transgenics, no attempts have been made to commercialize any transgenic food producing animals. In addition to technology, there are several factors that will impact the use of transgenic animals for food production. The first involves regulatory approval of meat or milk from genetically modified cattle. The authorities regulating genetically modified animals must address three factors;

1. safety of the food product for human consumption
2. environmental impact of the genetically modified animals and
3. welfare of the animals

conceptually, many of the modifications that might be considered to enhance production efficiency would not have any impact on the safety or quality of the food product. Since there are no wild bovine species, the transmission of modified genes into wild species is not a concern with cattle as it is with genetically modified plants, therefore, it is unlikely that genetically modified cattle would have a significant impact on the environment. The welfare of the animal could be a concern with some genetic modifications but could be easily evaluated.

Transgenic Animals for Human Therapeutic Production

A second application for genetically modified cattle is the production of human therapeutic proteins. Humans proteins that have been expressed in milk include human lactoferrin (van Berkel *et al.,* 2002), human alpha lactalbumin (Eyestone, 1999), human serum albumin (Behoodi *et al.,* 2001) and human bile salt stimulated lipase (Chen *et al.,* 2002). The mammary gland in dairy cows is an excellent protein production factory. Large quantities of very complex proteins can be produced and collected at very low cost.

Some research groups are using microchromosome transfer and gene targeting technologies to develop a line of genetically modifies cows that produce human polyclonal antibodies. To get rid of contaminating bovine antibody the bovine antibody genes are targeted with a knock out sequence to prevent expression. Antibodies are currently used for many different human clinical applications, including treatment of infectious disease, cancer, transplanted organ rejection, autoimmune diseases and for use as antitoxins. To make a human antibody product the genetically modified cows are immunized with a vaccine containing the disease agent. For example, a product could be made for treatment of *Staphlococcus aureus* infections acquired following hospitalization by immunizing the genetically modified cow with the *Staphlococcus aureus* bacterium. Following immunization the cow builds up an antibody response to the bacterium. To harvest the antibodies from the

cow, blood plasma is collected using a procedure that is similar to collecting plasma from human donors. The plasma is then processed to remove all contaminating bovine components so the final product is a human antibody that reacts to *Staphlococcus aureus* which can be injected into hospital patients to help them fight and infection.

Biotechnology in Animal Health

One important benefit from biotechnology is the diagnosis of livestock diseases, and genetically transmitted conditions which damage health and productivity. Biological techniques can also produce cheaper and more efficient drugs. In cases where a natural source material is prohibitively expensive, genetic engineering (in microbial of tissue culture systems) can be used to produce drugs of high value for humans or animals. Examples are insulin, human growth hormone and tissue plasminogen activator (used in treating heart disease).

Vaccines

Vaccines are used to stimulate an animal's immune system to produce the antibodies needed to prevent infection. Recombinant DNA technology has provided the means to produce large quantities of inexpensive vaccines, while a better understanding of the immune system has helped produce vaccines that do a better job of boosting the body's immune system. These engineered products are safer than traditional vaccines. Whereas conventional vaccines sometimes revert to virulent (disease causing) forms the new vaccines can be engineered to eliminate this threat.

Biotechnology is also producing an entirely new use for vaccines. They are being used to modulate hormones to increase growth rates, improve the efficiency of feed conversion, stimulate milk production, contribute to improved carcass quality and leaner meat, and enhance or suppress reproductive functions.

Korean scientists have developed a combined vaccine against pleuropheumonia, pneumonic pasteurellosis and enzootic pneumonia in swine. Molecular biology has been used to produce an improved vaccine to protect pigs from swine fever. In the Philippines, it has been used to develop an improved vaccine to protect cattle and water buffalo against hemorrhagic septicemia. This disease is the leading cause of death among these animals. The new vaccine gives improved protection at a very low cost. A field kit has also been developed, to diagnose this disease from nose swabs.

Diagnosis of Disease and Genetic Defects

Successful control of a disease requires accurate diagnosis. The ability to generate highly specific antigens by recombinant DNA techniques has made it possible for an increasing number of enzyme-linked immunosorbent assays (ELISA) to have the capacity to differentiate between immune responses generated by vaccination from those due to infection. This has made it possible to overcome one of the major drawbacks of antibody detection tests: the fact

that, because antibodies can persist in animals for long periods, their presence may not indicate current infection.

The advent of PCR has enhanced the sensitivity of DNA detection tests considerably. For example, PCR used in combination with hybridization analysis, has been shown to provide a sensitive diagnostic assay to detect bovine leukosis virus.

Other diagnostic techniques include nucleic acid hybridization and restriction endonuclease mapping. A good example of the specificity of nucleic acid hybridization is its application in distinguishing infections caused by *peste des ruminants* (PPR) virus from rinderpest, diseases whose symptoms are clinically identical and which cannot be distinguished antigenically with available serological reagents. This technique also allows comparison of virus isolates from different geographical locations.

Molecular epidemiology is a fast growing discipline that enables charaterisation of pathogen isolates (virus, bacteria, parasites) by nucleotide sequencing for the tracing of their origin. This is particularly important for epidemic diseases, where the possibility of pinpointing the source of infection can significantly contribute to improved disease control. Furthermore, the development of genetic probes, which allow the detection of pathogen DNA/RNA (rather than antibodies) in livestock, and the advances in accurate, pen-side diagnostic kits can considerably enhance animal health programmes.

DNA testing is also being used to diagnose hereditary weaknesses of livestock. One test identifies the gene which produces Porcine Stress Syndrome in pigs. Pigs with this gene tend to produce pale, poor-quality meat when they undergo the stress of transport or slaughter. Now that pigs with this gene can be identified, they can be excluded from breeding programs, so the gene will become less common.

DNA is also being used to diagnose a mutation of Holstein cattle that causes leucoyte adhesion deficiency. Cattle with this condition suffer diseases of the gum, tooth loss and stunted growth. They usually die before they are one year old. This test will identify carriers and eliminate them from breeding herds. Bulls used for breeding can be tested to make sure they are not carriers.

DNA technologies in animal nutrition and growth

Nutritional physiology

Applications are being developed for improving the performance of animals through better nutrition. Enzymes can improve the nutrient availability from feedstuffs, lower feed costs and reduce output of waste into the environment. Prebiotics and probiotics or immune supplements can inhibit pathogenic gut microorganisms or make the animal more resistant to them. Administration of recombinant somatotropin (ST) results in accelerated growth and leaner carcasses in meat animals and increased milk production in dairy cows. Immunomodulation can be used for enhancing the activity of endogenous anabolic hormones.

In poultry nutrition, possibilities include the use of feed enzymes, probiotics, single cell protein, and antibiotic feed additives. The production of tailor-made plant products for use as feeds and free from antinutritional factors through recombinant DNA technology is also a possibility.

Plant biotechnology may produce forages with improved nutritional value or incorporate vaccines or antibodies into feeds that may protect the animals against diseases.

Rumen biology

Rumen biology has the potential to improve the nutritive value of ruminant feedstuffs that are fibrous, low in nitrogen and of limited value for other animal species. Biotechnology can alter the amount and availability of carbohydrate and protein in plants as well as the rate and extent of fermentation and metabolism of these nutrients in the rumen contribution of introduced new strains.

Methods for improving rumen digestion in ruminants include the use of probiotics, which is the supplementation of animal feed with beneficial live microbes to improve the intestinal microbial balance for better utilization of feed and for good health. The added bacteria may improve digestion of feed and absorption of nutrients, stimulate immunity to diseases, or inhibit growth of harmful microbes. Transgenic rumen microbes (chapter 6) could also play a role in the detoxification of plant poisons or inactivation of antinutritional factors. Successful introduction of a caprine rumen inoculum obtained in Hawaii into the bovine rumen in Australia to detoxify 3-hydroxy 4(IH) pyridine (3,4 DHP), a breakdown product of the non-protein amino acid mimosine found in *Leucaena* forage is an example.

Genetic Engineering of Microorganisms of Interest to Agriculture

Introduction

In the context of genetic engineering targeting development of genetically modified organisms (GMOs), micro organisms of interest to agriculture represent a genetic resource. These microbes may find use as gene transfer systems or donors or recipients of desirable genes. Microbes functioning as gene transfer systems and as donors of genes, have already been discussed. The focus of this chapter is therefore on microbial recipients of transgenes.

Microorganisms play important roles in different sectors of agriculture, food processing, pharmaceutical industries and environmental management. Microbial processes are under the control of genes and there is need to continually improve and optimize their specific processes through genetic improvement. Traditionally this largely depended on the identification and selection of mutants with desirable characteristics. Recombinant DNA technology presents a number of benefits to this area, since specific metabolic pathways can be manipulated with more precision and completely new functions can now be engineered into the microbes. The following sections give some examples of microorganisms of economic importance that have been genetically modified through recombinant DNA Technology.

Genetically modified Microbes as Biopesticides and Biofertilizers

Biological control agents are especially targeted for genetic enhancement because of an increasing emphasis given to them in modern agriculture. Biological control represents an alternative to chemical pesticides which have been the object of much criticism due to their adverse impact on the environment and human health. There is therefore a need to develop safer and environmentally amenable control using existing organisms in their habitats. These organisms offer protection against a wide range of plant pests and pathogenic microbial agents without damage to ecosystems.

In order for biological control agents to be effective in plant disease management, they must be effective, reliable and economical. To meet these conditions superior strains are often required. In this case the existing attributes of the biocontrol agents can be genetically manipulated to enhance their biocontrol activity and expand its spectrum.

The foreign genes used for transforming biological control agents can be integrated into the host genome or plasmid. To express a heterologous gene in fungi or bacteria, the regulatory region of this gene must be modulated in promoter and terminator exchange in order to optimize the activity of the inserted gene in the new host. Addition of specific genes known to confer biocontrol activity may enhance or improve biocontrol capacity of organisms deficient in the genes targeted for transfer. The majority of rhizobacterial with biocontrol activity produce chitinases.

Free living bacterial associated with plants have been targeted to enhance their capacity either as soil inoculants or as biocontrol agents of plant pathogens. Studies on microbes capable of enhancing plant growth have concentrated on the rhizosphere whereas those on biocontrol target both rhizosphere and phylloplane. Several important rhizobacteria including *Rhizobium melliloti* and *Pseydomonas putidrii,* both of which are excellent root colonizers lack the ability to synthesize chitinases. Introducing genes encoding chitinases into their genome have enabled them to provide protection against plant pathogenic fungi. These two bacteria are good targets because of unique beneficial characteristics they confer. *Rhizobium* is a symbiotic bacterium which stimulates formation of root nodules in legumes involved in fixing atmospheric nitrogen. Many pseudomonads in the rhizosphere environment produce siderophores which chelate iron ions, thereby increasing iron uptake by plants. The genetically modified commercial strain (RMBPC-2) of *Sinorhizobium melliloti* has added genes that regulate nitrogenase enzyme (nitrogen fixation) from the plant to the bacterium.

Trichoderma species are widely represented in soils and are antagonistic to other fungi. *T. harzianum,* in particular is a strong rhizosphere colonizer which is also able to parasitize plant pathogenic fungi. It establishes tight physical contact with hyphae of target fungi with the aid of binding lectins. Several extracellular enzymes including chitinases, glucanases, lipases and *proteases* produced by *Trichoderma* species has been improved with the transfer of chitinase genes, notably from *Serratia marcessens.*

Agrobacterium radiobacter strain k84 protects plants against crown gall caused by *A. tumefaciens* strains carrying Ti-plasmid of the nopaline type. Protection conferred by *A. radiobacter* strain k84 is due to agrocin 84, an adenine nucleotide derivative, when taken up by *A. tumefaciens,* inhibits DNA synthesis, resulting in cell death. *A radiobacter* has an additional negative effect on soil pathogens by being a very effective rhizosphere colonizer. Although *A. radiobacter* strain 84 has been widely used commercially for a long time, there was concern about its long term effectiveness as a biocontrol agent. This is because the gene encoding agrocin is carried on a transmissible plasmid, which can be transferred by conjugation to *A. tumefaciens.* In the event of agrocin-encoding plasmid transfer, receipient *A. tumefaciens* strains will not be subjected to biocontrol by *A. radiobacter* strain k84. This concern was addressed by modification of the agrocin-encoding plasmid to prevent its transfer to *A. tumefaciens*. The ensuing genetically engineered strain, known as *A radiobacter* strain K1026, is a transgenic organism approved for use as a pesticide.

Bacillus thuringiensis (Bt) has been used as a biopesticide for a long time, however it has the disadvantage of fast degradation in sunlight. Different cry genes encoding the Bt toxin have been cloned and introduced into another bacterium, *Pseudomonas flourescens.* The transgenic *P. flourescens* strains are killed and used as a more stable and persistent biopesticide compared to the *B. thuringiensis* sprays.

Baculoviruses are also being manipulated to be effective biopesticides against insect pests such as corn borer, potato beetle and aphids.

Microbes for Enhancing the Use of Animal Feeds

Animal digestive tracts harbor beneficial microflora that aid in the digestability of various feeds. However, the function of these microbes is easily affected by the unfavorable conditions within the gut, such as acidity and antibiotics used to treat pathogenic microbes. Examples of gut microbes that have been genetically modified include; *Prevotella ruminicola* with a tetracycline resistant gene, cellulolytic rumen bacteria with acid tolerance, hind gut bacteria with cellulose activity, rumen bacteria transformed with genes to improve protein yield and yeast (*Saccharomyces cerevisiae)* containing a transgene from a closely related *Saccharomyces diastaticus,* allowing it to better increase the digestibility of low-quality roughage in conventional feeds. The major limitation to the use of these engineered organisms has been their establishment in the appropriate regions of the gut. Some organisms are being used as beneficial supplements in animal feeds. These are called probiotics and their use aims at improving digestion of feed and absorption of nutrients, stimulate immunity to diseases and inhibit growth of harmful microbes.

In the improvement of silage, strains of the bacteria *Lactobillus planetarium* are being developed which increase the lactate content and reduce the pH and ammonia-N content.

Microbes are being used as bioreactors for the production hormones and other substances that enhance animal size, productivity and growth rates. Synthetic hormone bST (bovine

somatotropin) was among the first innovations available commercially. It can increase milk yield by as much as 10 to 15 percent in lactating cows. Current development efforts are looking at a wide spectrum of genes that affect growth and production within the animal.

Genetically Modified Microbes in Food Processing

Many microbes are being manipulated with the objectives of improving process control, yields and efficiency as well as the quality, safety and consistency of bioprocessed products. Modifications target food enzymes, amino acids, peptides (sweeteners and pharmaceuticals) flavors, organic acids, polysaccharides and vitamins. Classical example is the production of the recombinant cheese making enzyme, chymosin in bacteria. Its use was approved in 1990 in the USA, and now 80% of US cheese is produced using this product.

Genetically Modified Microbes in Bioremediation

Microbes are widely used in cleaning up pollution such as oil spills, agricultural and industrial wastes by degrading them into less toxic compounds. Some bacteria are being used as "bioluminescensors" that give luminescence in response to chemical pollutants e.g. the mercury resistance gene *mer* expressed in some bacteria can light up signaling the presence of even very low levels of mercury in the environment.

A modified bacterium, *Rhodopseudomonas capsulate*, has the ability to grow rapidly in simple synthetic media. It is being used in advanced swine waste treatment plants in both Japan and Korea. Short chain fatty acids, one of the main sources of the bad odor of swine wastes, decreased dramatically after treatment. The residue after treatment can be used as a safe organic fertilizer.

GMOs Detection Methods

Introduction

Different stackholders involved in the development, use and regulation of genetically modified organisms (GMOs) do at some point need to monitor and verify the presence and the amount of GMOs in agricultural crops and in products derived thereof. This need has generated a demand for analytical methods capable of detecting, identifying and quantifying either the unique DNA sequences introduced or the protein(s) expressed in transgenic plants. Thus comprehensive GMO analysis techniques consists of three steps: detection, identification and quantification.

1. ***Detection (screening of GMOs):*** The objective is to determine if a product contains GMO or not. For this purpose, a screening method can be used. The result is a positive/negative statement. Analytical methods for detection must be sensitive and reliable enough to obtain accurate and precise results.

2. ***Identification:*** The purpose of identification is to reveal how many different GMOs are present and if they are authorized or not. Specific information (i.e. details on the molecular make-up of the GMOs) has to be available for the identification of GMOs.
3. ***Quantification:*** If a food product has been shown to contain (one or more) authorized GMOs, then it becomes necessary to assess compliance with the set threshold level regulation by the determination of the amount of each of the GMOs present in the individual ingredients from which the food item has been prepared (e.g. maize flour).

GMO detection by phenotypic characterization

Phenotypic characterization is possible if the gene targeted allows for determination of absence or presence of a specific trait. Detection methods using this approach are referred to as bioassays. This approach can be used to test for the presence or absence of herbicide resistance transgenes. This test involves germinating seeds in the presence of the herbicide of interest. Herbicide assays are considered to be accurate and inexpensive. Controls, including seeds with or without the trait targeted should be included in all samples tested. Herbicide assays are available for Roundup-Ready crops. Typically a test of 400 seeds is used. The accuracy is dependent on the germination: the higher germination the higher is the confidence level of the test. Only viable seed or grain can be tested (no processed products), and each test requires seven days to complete. The potential error of accuracy increases as the germination level of the sample decrease. Furthermore, bioassays require separate tests for each trait in question and at present the tests will not detect non-herbicide tolerance traits. Therefore, the tests are only of limited value for inspection authorities.

Molecular Detection of GMOs

Methods that target the inserted DNA and its expressed proteins have been developed and are widely used for detecting GMOs. This is because the target DNA can be purified and amplified by polymerase chain reaction (PCR) [Box 1]. Another advantage associated with working with DNA is that there is usually a linear relationship between quantity of GMO and the inserted DNA carried in the nucleus, thus it can be used to quantify the amount of GMO material present in a sample.

Protein-based methods rely on a specific binding between the protein and an antibody. The antibody recognizes the foreign molecule, binds to it, and in GMO detection assays the bound complex is successfully detected in a chromogenic (color) reaction. This technique is called ELISA (Enzyme Linked ImmunoSorbent Assays). The antibody needed to detect the protein cannot be developed without access to the purified protein. This protein can be purified from the GMO itself, or it can be synthesized in a laboratory if the composition of the protein is known in detail.

The Polymerase Chain Reaction (PCR)

The PCR reaction allows the million-fold amplification of a specific target DNA fragment framed by two primers (=synthetic oligonucleotides, complementary to either one of the two strands of the target sequence). In principle, the PCR is a multiple-step process with consecutive cycles of three different temperatures, where the number of target sequences grows exponentially according to the number of cycles. In each cycle the three temperatures correspond to three different steps in the reaction. (*Refer to diagrams in the power point presentation).*

In the first step, the template, i.e. the DNA serving as master-copy for the DNA polymerase is separated into single strands by heat denaturation at ~94^{o}C.

In the second step, the reaction mix is cooled down to a temperature of 50-65^{o}C (depending on the GC content) to allow the annealing of the primers to the target sequence. Primer hybridization is favoured over DNA-DNA hybridization because of the excess of primers molecules. However, the annealing process is uncontrolled and can give rise to a large number of mismatched DNA duplexes,

In the third step, the annealed primers are extended usually by a *Thermus aquaticus* (Taq) polymerase at the optimum temperature of 72^{o}C. With the elongation of the primers, a copy of the target sequence is generated).

The cycle is then repeated 20 to 50 times, depending on the amount of DNA present and the length of amplicon (= amplified DNA fragment).

PCR Based GMO Detection

For routine GMO screening purposed one should focus on target sequences that are characteristic for the group to be screened. Genetic control elements such as cauliflower mosaic virus 35S promoter (P-35S) and the *Agrobacterium tumefaciens* nos terminator (nos3') are present in many GMOs currently on the market. The first GMO screening method is based on the detection of the P-35S and nos3' genetic elements. However, a few approved GMOs are not screenable/detectable with the P-35S or the nos3' primers and additional target sequences are needed to guarantee a complete screening procedure. A further aspect is the choice of primers that allow the detection of as many variants as possible of a GMO marker, e.g. there are at least 8 variants of P-35S used in GM crops. It should be stressed however, that the detection of these generic GMO markers only indicates that the analysed sample contains DNA from a GM plant, but does not provide information on thespecific trait that has been engineered in the plant.

Most PCR-based GMO detection methods include a positive control primer set, which is specific for a gene that is present naturally in all varieties of the applicable species. This reference is a species-specific gene, such as the lectin gene is soybean or the invertase gene in maize. If a strong signal is not obtained with the positive control primer set, then there may be problems with the integrity or purity of the DNA. On the other hand, if the DNA is

detectable, the samples can be screened using the general genetic elements which cater for multiple varieties of GMO DNA. Furthermore, if positive results from this initial screening are obtained, additional confirmation using tests which screen for specific genes or constructs are most common in GM crops is carried out. This three-step approach ensures that results obtained have been confirmed using multiple screening systems. This approach applies to situations where sequence material or reference data are available.

Confirmatory assays

Confirmation/verification of the identity of the amplicon is necessary to ensure that the amplified DNA is really corresponding to the chosen target sequence and is not a by-product of un-specific binding of the primers. Several methods are available for this purpose. Gel electrophoresis is the simplest approach to control if the PCR products have the expected size. However, there is a risk that an artifact having the same size of the target sequence may have been amplified. Therefore, the PCR products should additionally be verified for their restriction enzyme profile. Even more reliable is a Southern blot assay, whereby the amplicon is separated by gel electrophoresis, transferred onto a membrane and hybridized to a specific DNA probe. Another possible control is to subject the PCR product to a second round of PCR cycle in a technique that is called nested PCR. Here, two different sets of primers – an outer and an inner (nested) pair – are being used within the target region in two consecutive rounds of PCR amplifications. This strategy reduces substantially the problem of un-specific amplification, as the probability for the inner pair of primers of finding complementary sequences within the non-specific amplification products of the outer pair is extremely low. The most reliable way to confirm the authenticity of a PCR product is its sequencing. However, only few laboratories are equipped to carry out this approach routinely.

Protein-based methods: Immunoassay

Immunoassay is the current method for detecting and quantifying a target protein associated with genetic modification. It can be used for qualitative and quantitative measurements over a range of concentrations. Different types of immunoassays including enzyme-linked immunosorbent assay (ELISA), dipstick and lateral flow procedures are available for use in the field and in the laboratory.

ELISA

In ELISA, the protein-antibody reaction takes place on a solid support and the protein and antibody react to produce a complex. This complex is usually visualized by adding a second antibody linked to an enzyme. When the substrate for the enzyme is added, a colored product is formed. The intensity of the color can be measured photometrically and used for quantitative assessments.

Some ELISA plates are supplied with calibration or known concentration of target protein in solution and a negative control defined by the absence of the target. The standards

will exhibit distinctively different intensities of a given color at the different concentrations of target molecules provided. By comparing the intensity of color of the sample tested for GMO target molecules with that of the standards, it is possible to work out the concentration range of the target. These immunoassay measurements are qualitative.

Quantitative measurements can however be obtained by using a microplate reader which measures the absorbance of all samples and standards at the same time. The detection limit is less than 0.01 percent.

Lateral flow strips and dipsticks

Paper strips or plastic paddles on which antibody is captured are also used to detect proteins targets of GMOs. The strip is dipped in vials containing solutions of the sample to be tested. Each dip is followed by rinsing. The positive reaction is a color change in the vial. Recent improvements of the 'dip stick' have produced lateral flow strips in which reagents are transported through nylon membrane by capillary action. Antibodies specific to the target protein are coupled to a colored reagent and one incorporated into the lateral flow strip. When the strip is brought into contact with a small amount of the sample containing the target protein, an antibody-antigen complex is formed with some of the antibody. The membrane contains two capture zones, one for the bound protein and the other for the colored reagent. A colored band appears in the capture zone corresponding to the bound antibody-protein complex and colored reagent. Appearance of a single colored band in the membrane is a negative test for the presence of the protein targeted. The presence to two bands represents detection of the target.

PCR Methods for GMO Identification

Following a positive result from a GMO screening exercise the next step is the unequivocal identification of the GM event(s) involved. This can be achieved by one of the following PCR strategies.

Gene specific PCR. This is a PCR system targeting one element of the transgenic element. It is less specific and only useful if the genetic element is present in only one event.

Constructive specific PCR. Sometimes its sufficient to only identify GMOs using PCR system designed for the amplification of the junction between different elements of the transgenic insert.

Event specific PCR. This is the most specific GMO identification strategy. It is designed to amplify the junction between the transgenic insert and the host DNA (the so called fragments)

Thus there is need for a continuous survey of all data available on GMOs – especially the introduced genetic elements and their integration sites, not only for GM products approved for market release but also for any other GMO released for field trials worldwide. This can guarantee a complete comprehensive monitoring, detection and identification of GMOs.

Quantitative PCR

In the event that there is positive test for GMO content in a sample, it is important to quantify the amount to test for compliance with specific threshold levels of GMOs established by biosafety regulations. The typical approach to quantification utilizes one or more of the broad-spectrum primer set that recognizes common transgenic elements in GMOs. However, since different transgenic events contain these common elements in different numbers, accurate determination of GMO content cannot rely on the use of these common sequence elements. Quantification based on event-specific primers is therefore the most accurate means of obtaining quantitative results on GM content.

Use of Conventional PCR quantification

One possibility for DNA quantification using conventional PCR is competitive PCR. In competitive PCR, one primer pair is used to amplify both the target GMO DNA and a synthetic DNA fragment of known concentration in the same reaction mixture. The second fragment, which is a different size to the GMO target DNA, is called the competitor. By conducting a series of experiments with varying amounts of the synthetic DNA, it is possible to determine the amount of target GMO DNA. In situations when the PCR products are equal in intensity as determined visually following gel electrophoresis, the initial amounts of target GMO DNA equal the initial amounts of synthetic DNA. Competitive and double competitive PCR methods are semi-quantitative as a standard is required for comparison. In these cases the standard is the known amount of synthetic DNA. Consequently, the results will only indicate a value below, equal or above a defined concentration of the standard.

Real-time PCR for GMO quantification

Another strategy of the second group that improves accuracy, specificity and throughput of quantitative PCR is "Real-time PCR". This technique was originally developed in 1992 and is rapidly gaining popularity due to the introduction of several complete real-time PCR instruments and easy-to-use PCR assays. A unique feature of this PCR technique is that the amplification of the target DNA sequence can be followed during the whole reaction by indirect monitoring of the product formation. Therefore, the conventional PCR reaction has to be adapted in order to generate a constant measurable signal, whose intensity is directly related to the amount of amplified product. Real-time detection strategies rely on continuous measurements of the increments in fluorescence generated during the PCR reaction. The number of PCR cycles necessary to generate a signal that is significantly and statistically above noise level is taken as a quantitative measure and is called cycle threshold (C_t) (figure 7.1A). As long as the C_t value is measured at the stage of the PCR where the efficiency is still constant, the C_t value is inversely proportional to the log of the initial amount of target molecules (figure 7.1B).

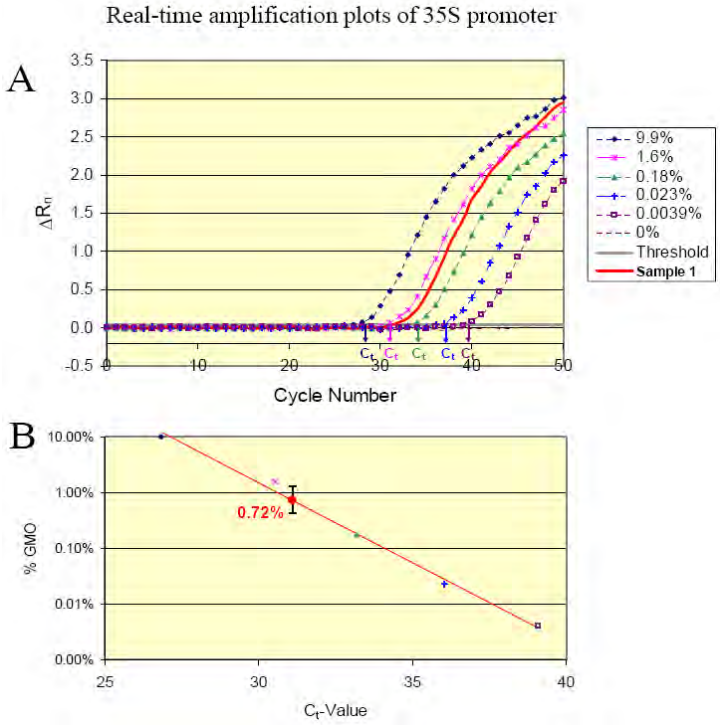

Fig 7.1. Real Time PCR. **A)** Diagram showing the accumulation of the target analyte 35S promoter at six different ratios of GMO/non-GMO material (% w/w). PCR product formation is visualized in real time by taking fluorescence measurements (R_n) at each cycle. The initial template concentration determination is based on the threshold cycle (C_t), i.e. the PCR cycle at which fluorescence is first detected statistically significant above background. C_t is inversely proportional to the log of the number of target copies present in the sample. **B)** Linear regression diagram showing the logaritmic relation between the GMO/non-GMO ratios and the C_t values.

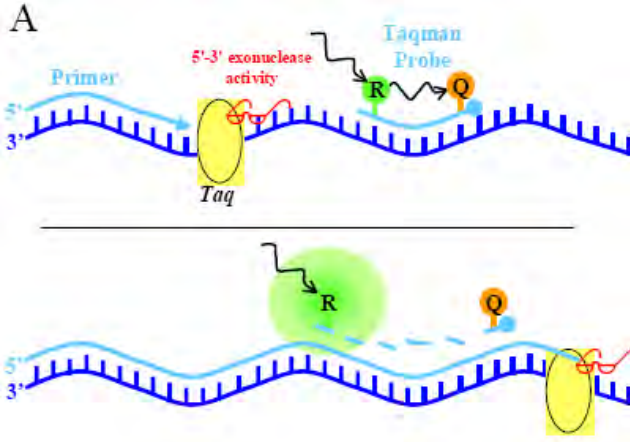

Fig 7.2. Amplicon Specific TaqMan probes used to monitor the PCR product formation in real-time (see text for details)

One of the most popular assays for real-time PCR is the Taqman® or 5'-exonuclease assay, which employs a fluoregenic probe consisting of an oligonucleotide with both a reporter (R) and a quencher (Q) dye attached (TaqMan® probe, see Fig 7.2). when the probe is intact the reporter fluorescence is quenched by the proximity of the quencher dye. Due to its target-specific sequence, the probe anneals specifically to the amplification product (target DNA) between the forward and the reverse primers. If hybridization has occurred, the 5'-3' exonuclease activity of the Taq polymerase cleaves the internal probe during the extension step of amplification. The cleavage reduces the quenching effect and the fluorescent signal of the reporter dye becomes a measure of the amount of amplification product generated (Fig 7.1A). Because the development of the fluorogenic reporter signal takes place only if both the PCR primers and the TaqMan® probe anneal to the target DNA, the specificity of real-time PCR detection is considerably higher than that of conventional PCR. The relative quantification of the target gene is made possible by preparing a standard curve from known quantities of an additional endogenous gene and extrapolation from the linear regression line.

Limits of quantification by PCR and Sampling

Although 0.01 percent is the limit of detection using PCR, quantitative measurement is not possible at this level. This is because the number of GMO targets in preparation derived from a sample containing 0.01 percent GMO material will be in the range 1 to 4 or more. These preparations will produce GMO target measurements that are more influenced by statistical variation associated with sampling than by actual GMO content in the sample. As a result, most laboratories set the limit of quantification by PCR 10 times higher (0.1%) to avoid measurement problems near the limit of detection.

Irrespective of the analytical method selected for GMO detection, correct sampling is critically important. Samples must therefore be taken in a manner that ensures that they are statistically representative of the larger volume or quantum of material. The sample size will therefore depend on the detection level required and what is being detected.

The International Seed Testing Agency (ISTA) has produced sampling guidelines. For example:

- 100 plants give a 95 percent confidence limit for detecting a 3 percent contamination level.
- 200 plants give a 95 percent confidence limit for detecting a 1.5 percent contamination level
- 300 plants give a 95 percent confidence limit for detecting a 1 percent contamination level.

The working sample which is a sub-sample of the overall sample should also be properly determined. According to ISTA methods the working seed sample should contain a minimum of 3000 seeds.

Alternative Techniques for GMO Analysis

Chromatography and Near Infrared Spectroscopy

Where the composition of GMO ingredients, e.g. fatty acids or triglycerides is altered, conventional chemical methods based on chromatography near infrared spectroscopy can be applied for detection of differences in the chemical profile. This has been demonstrated with oils deriving from GM canola for which high performance liquid chromatography (HPLC). Triglyceride patterns and content can be compared between GM and Non GM samples. However, it must be stressed that this methodology is only applicable when significant changes occur in the composition of GM plants or derived products. Moreover, it is a qualitative detection method rather than a quantitative method. Low additions of, e.g., GM canola oil with an altered triglyceride composition to conventional canola oil will most probably not be detected, also considering the natural variation of ingredient patterns.

Microarrays

Microarrays technology (DNA chip-technology) has been developed in recent years for automated rapid screening of gene expression and sequence variation of large number of samples. Microarrays technology is based on the classical DNA hybridization principle, with the main difference that many (up to thousands of) specific probes are attached to a solid surface. Different formats are known, e.g. macroarrays, microarrays, high-density oligonucleotide arrays (gene chips or DNA chips) and micro-electronic arrays.

Microarrays allow analysis of expression patterns of thousands of genes within the confines of one experiment. Arrays are direct descendents of DNA gel-blot (Southern) based assays that exploit interactions between complimentary strands of DNA. The addition of a solid glass substrate, precision robotics, and the use of fluorescence provide expression arrays with increased precision, speed and scale over their filter-and radioactivity-based cousins. Although transcript monitoring is currently the most popular use for arrays, they have been successfully utilized in fields ranging from mutation detection to evolutionary sequence analysis. Micro-arrays can be constructed using either PCR-amplified cDNAs or oligonucleotides.

Some GMO chip kit detects species-specific DNA of plants and viruses, generally used genetic construction elements, and specifically introduced genetic modifications for the identification of approved and non-approved plant varieties. For example, the GMO chip version “The European” detects specific DNA from soybean, maize, oilseed rape, rice, CaMV (species) and the following GMOs: RR-soybean, Maximizer Bt 176 maize, Bt11 maize, Yieldgard Mon810 maize and Bt-Xtra maize. In addition, GMO chip allows screening for all GMOs with the CaMV 35S promoter, Nos-terminator, bar-gene and pat-gene.

As microarrays technology has expanded, quantitative comparison of data within and across microarray platforms has proven difficult. The main reasons for this are a lack of universal references and the variety of data analysis methods in use. The microarray, in

principle, enables the detection, identification and quantification of large numbers of GMO varieties present in a sample in one single assay. Furthermore, microarrays are very flexible, as new varieties can be included in the screening procedure by adding additional sequences to the array.

Genes of Interest to Agriculture

Introduction

The field of field production has benefited immensely from recombinant DNA technology. Transgenic crops with novel agronomic and quality traits have developed and are grown in many developed and developing countries. For a detailed account on the nature and extent of utilization of the various GM crops, one can consult online databases such as AGBIOS (http://www.agbios.com) FAOBioDeC (http://www.fao.org/inventory.admin/dep/default.asp). ThAGBIOS website includes details of the transgenes, the science underpinning the traits and environmental and food safety issues. The FAOBioDeC database is an online inventory of biotechnologies in developing countries. It covers both genetically modified and non-GM technologies. Surveying information in these and similar databases it is possible to see what genes have been used in generation transgenic crops and which ones are still being developed.

Genes that seem to be of much interest to both the public and private sectors are those that confer resistance to biotic and abiotic stresses as well as quality traits. Comparisons of attainable and actual yields demonstrate that most crops are at best only reaching 20% of the genetic potential for yield. The reduction in yield are attributed to both biotic (e.g. pests, pathogens and weeds) and abiotic stresses. Out of a $1.3 trillion annual food production capacity worldwide, the biotic stresses caused by insects, diseases and weeds cause 31-42% loss ($500 billion), with an additional 6-20% ($120 billion) lost post harvest to insects and to fungal and bacterial rots. Crop losses due to pathogens are often more severe in developing countries (e.g. cereals 22%) when compared to crop losses in developed countries (e.g. cereals 6%). Weeds are also a major and continuing biotic constraint affecting cropping systems worldwide. Another 6-20% ($120 billion) is estimated to be lost to abiotic causes (drought, flood, frost, nutrient deficiencies, various soil and air toxicities). One of the most significant abiotic stress reducing crop yields is water stress, both water deficit stress (drought) and excess water stress (flooding, anoxia). It is in this context that the need arises to develop crops which are more resistant to biotic and abiotic stresses.

Genes for Resistance to Biotic Stresses

Herbicide Resistance Genes

Glyphosate herbicide tolerance: The genetically modified glyphosate resistant crops contain a gene encoding the enzyme 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS). The EPSPS gene was originally obtained from a strain of the soil inhabiting bacterium.

Agrobacterium tumeraciens. EPSPS allows plants to survive the otherwise lethal effects of the herbicide glyphosate. The EPSPS is an important part of shikimate biochemical pathway which is required to produce aromatic amino acids plants needs to grow and survive. Conventional plants treated with glyphosate cannot produce the aromatic amino acids. Glyphosate binds to and activates EPSPS.

Glufosinate ammonium herbicide tolerance

Glufosinate ammonium is the active ingredient in the phosphinothricin herbicides. Glufosinate chemically resembles amino acid glutamate and functions by inhibiting the enzyme glutamate synthase which converts glutamate to glutamine. Glutamine synthesis is also involved in the ammonia detoxification of glufosinate resulting in reduced glutamine levels and increases in ammonia concentration. Elevated levels of ammonia damage cell membranes and impair photosynthesis. Glufosinate tolerance is the result of introducing a gene encoding the enzyme phosphinothricin-aan-acetyl transferase (PAT). The gene was originally obtained from the soil actinomycete Streptomyces hygroscopiens. The PAT enzyme catalyses detoxification of phosphinothricin.

Sulfonylurea herbicide tolerance

Sulfonyl urea herbicides, such as triasulfuron and metasulfron-methyl target the enzyme acetolactate synthase (ALS), thereby inhibiting the biosynthesis of the branched chain animo acids valine, leucine and isoleucine. It resuts in accumulation of toxic levels of alpha-ketoglutarate. In addition to the native ALS gene, herbicide tolerant crops contain the ALS gene from a tolerant line of Arabadopsis thaliana. This variant ALS gene differs from the wild type by one nucleotide and the resulting ALS enzyme differs by one amino acid from the wild type ALS enzyme.

Oxynil herbicide tolerance

Oxynil herbicides and bromoxynil are effective against broad leaf weeds. Transgenic herbicide resistant crops contain a copy of the bxn gene isolated from the bacterium Klebsiella pneumoniae. The gene encodes a nitrilase which hydrolyzes oxynil herbicides to non-phytotoxic compounds.

Insect Resistance

Among the insect pests, Lepidoptera represent a diverse and important group. Most insect-resistant transgenic crop varieties developed so far for the control of Lepidoptera, predominantly using transgene cassettes including toxin-producing Cry-type genes obtained from strains of the soil bacterium Bacillus thuringiensis (Bt). The proteins bind to specific sites on the gut lining in susceptible insects. The binding disrupts midgut ion balance which eventually leads to paralysis, bacterial sepsis and death. In addition to Bt genes, protease inhibitors, neuropeptides and peptide hormones that control and regulate the physiological

processes of several insect pests have become candidates for developing insect resistant crops. Other biocontrol toxins currently studies are chitinases, lectins, alpha-amylase inhibitors, cystatin and cholesterol-oxidase and glucosidase inhibitors.

Resistance to Pathogens

Among the disease causing organisms, viruses have received a lot of attention in as far ad the development of transgenic crops is concerned. This has been possible since the discovery of pathogen derived resistance, where the expression of a viral protein (e.g. coat protein, replicase, helicase enzyme etc) in a transgenic plant renders that plant resistant to the virus. As a result many viral genes have been cloned and used to transform crops. Genes encoding chitinases and glucaneses have been used to generate plants resistant to fungal and bacterial pathogens respectively. Other strategies for conferring resistance to pathogens in transgenic crops include genes for phytoalexins which are involved in pathogen induced localization or infection and R genes (resistance genes) being identified as responsible for defense mechanisms in plants.

Genes for Resistance to Abiotic Stresses

So far there are no commercialized transgenic crops with resistance to abiotic stresses such as drought, heat, salinity and frost. However, a number of approached are being developed to tackle these stress factors in crops.

Genes for Improved Quality Traits

Modified Flower Colour

Many flowers including carnations, roses, lilies, chrysanthemums and gerberas, all of which are important in global flower trade, do not produce the blue pigment delphinidin. Transgenic carnation lines with unique violet/mauve color have been developed. The genes of interest here include structural and regulatory genes of the flavanoid biosynthetic pathway.

Delayed Fruit Ripening and Increased Shelf life

Genes encoding an enzyme which degrades 1-aminocyclopropane 1-carboxylic acid (ACC), an ethylene precursor, and those encoding polygalacturonase (PG) have been suppressed in some transgenic plants. Suppression is accomplished by inserting a truncated or anti-sense version of the PG gene. Reduced ACC results in delayed fruit ripening while that of PG decreases the level of cell wall breakdown and hence delays fruit softening and rotting.

Modification of oil composition

Oil seed rape and soybean have been modified to increase the content of oleic acid in particular. The modified oils are lower in saturated fat and have greater heat stability than oils from the corresponding unmodified crops. In unmodified crops the FAD2 gene encoding a

desaturase converts C18:1 (oleic acid) to C18:2 and C18:3. In the modified crop a mutant FAD2 gene blocks expression of the active desaturase and as a result there is accumulation of oleic acid.

Modified Vitamin and Mineral Profiles

Vitamins and minerals are essential components of the human diet and dietary deficiencies of these nutrients can have tragic effects. In addition to fortification and supplementation strategies for alleviating these deficiencies, transgenic crops with elevated and bioavailable vitamins and minerals are being developed. Here the strategy is to express the genes responsible for the production or accumulation of the concerned nutrient in the edible parts of the plant. Thus promoters and sequences that target the expression of the genes of interest are equally important. In order to improve vitamin A production in rice the genes encoding phytoene synthase and phytoene dasaturase have been express in the endosperm. To improve iron accumulation and bioavailability in rice, genes such as ferritin synthase from soya (Fe storage), metallothionein (cystein rich storage protein, improve Fe sabsoption) and a heat stable phytase gene (degrades phytic acid which inhibits Fe absorption) have been expressed in the rice endosperm.

In: Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues. Chowdhury MKA, Hoque MI and Sonnino A (Eds.). pp. 51-105.

Chapter 2: Ecological Aspects of Biosafety

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Introduction

The debate on GMOs is no longer whether they should be released or not; it has moved to how and why they are being put to use. The GMOs are here to stay; the challenge is to use them to our advantage without jeopardizing the safety of the environment and human health. The acreage being planted to GMOs is increasing at exponential rates. In 1999, 11 million ha were planted to GM crops. By 2001, the area had increased to 44 million ha up to 114.3 million ha in 2007. GM crops were grown in 23 countries in 2007 (12 developing countries and 11 industrial countries). The total GM crop area was planted to four main crops: soybeans, maize, cotton and oilseed rape. Size of farm has not been a factor affecting use of the technology. Both large and small farmers have adopted GM crops. Size of operation has not been a barrier to adoption. In 2007, 12 million farmers were using the technology globally, 91% plus of which were resource-poor farmers in developing countries notably Argentina, Brazil, China, India and Paraguay (James, 2007).

GMOs carry exogenous genes or sequences. There is potential for these genes to affect gene expression in the plant and also for the product of this gene expression to interact with compounds in the plant. GMOs are introduced into ecosystems, and they will invariably interact with other organisms. The purpose of this module is to provide the necessary background information on ecology and evolution needed to analyse and understand the consequences of introducing GM organisms into the environment, as well as to show that many areas in ecology can benefit from research tools based on applications of molecular genetics and biotechnology. These tools include investigations into population biology and evolution, and conservation and use of genetic resources for both human requirements and environmental protection.

Introduction to Environmental Biosafety

Meeting the food needs of the world's growing population while reducing poverty and protecting the environment is a major global challenge. It is essential to take environmental concerns into account in order to develop technological solutions that are sustainable in the long run. Meeting these demands will require agricultural productivity increases and product diversification to improve the livelihoods of the poor, protect the environment, and ensure broad-based economic growth (UN Millennium Project 2005). Plant genome sciences, and plant biology as a whole, are vital enterprises that can contribute significantly to human health, agriculture, energy security, and environmental stewardship (The Academies of

Sciences, 2007). Promoting agriculture alone will not be enough to massively reduce poverty, but it can contribute to produce faster growth, reduce poverty, and sustain the environment (World Bank, 2007). Modern biotechnology is an important tool for crop improvement and novel uses of plants, animals, and microorganisms. Biotechnology is not a panacea, but a resource that can be useful when combined with adaptive research capacity (FAO, 2004).

Environmental benefits and concerns related with GM crops

Agriculture of any type – subsistence, organic or intensive – affects the environment, so it is natural to expect that the use of new genetic techniques in agriculture will also affect the environment. Releasing transgenic crops into the environment may have direct or indirect effects including: gene flow -gene transfer to wild relatives or conventional crops-, weediness, trait effects on non-target species, pest resistance, and other unintended effects. These risks are similar for transgenic and conventionally bred crops. Although scientists differ in their views on these risks, they agree that environmental impacts need to be assessed on a case-by-case basis and recommend post-release ecological monitoring to detect any unexpected events (ICSU, 2003). Transgenic crops may also entail positive or negative indirect environmental effects through changes in agricultural practices such as pesticide and herbicide use, and cropping patterns.

Some GM micro-organisms can be used in the environment as biological control agents or for bioremediation of environmental damage (e.g. oil spills), and their environmental effects should be assessed prior to release. One of the most significant environmental benefits of GM crops is the dramatic reduction in pesticide use (varying between crops and the introduced trait). Additionally, no-till, or conservation till cultivation systems are used, contributing to a reduction in fuel use in soil cultivation. The cumulative global impact of GM technology on farm income, pesticide usage, and greenhouse gas emissions among other indicators, has been studied and documented. Among the significant benefits for the farmers derived from the use of GM crops are higher crop yields, reduced farm costs, increased farm profit, and improvement in health and the environment (Brookes & Barfoot, 2008; 2006; NRC-CEI, 2002; Traynor *et al.*, 2002). The studies show that up to 2006 there have been substantial economic benefits at the farm level, amounting to a cumulative total of US$27 billion derived from enhanced productivity. Since 1996, there has been a 24.6% reduction in the environmental impact, and a 22.9% decrease in the volume of insecticides applied; the majority of the environmental benefits associated with lower insecticide and herbicide use have been for developing country farmers. The vast majority of these environmental gains have been from the use of GM IR cotton and GM HT soybeans (Brookes & Barfoot, 2008). Over the period 1996 to 2006 the cumulative permanent reduction in fuel use is estimated at 5,821 million kg of carbon dioxide (arising from reduced fuel use of 2,120 million litres). The carbon dioxide savings from reduced fuel consumption since the introduction of GM crops are equal to removing 2.58 million cars from the road for one year, and the additional probable

soil carbon sequestration gains in 2006 were equivalent to removing nearly 6.02 million cars from the roads (Brookes & Barfoot, 2008).

The “first” generation of GM crops has proven their ability to lower farm-level production costs. Now, research is focused on “second-generation” GM crops that will feature increased nutritional and/or industrial traits. These crops will have more direct benefits to consumers, and include: rice enriched with iron and provitamin A, potatoes with higher starch content, edible vaccines in maize and potatoes, crop varieties able to grow in stress conditions, healthier oils from soybean and canola (Lemaux, 2008; AgBios, 2002). Despite their potential, there is a multitude of concerns about the impact of GM crops on the environment. Key issues in the environmental assessment of GM crops are putative invasiveness, vertical or horizontal gene flow, other ecological impacts, effects on biodiversity and the impact of presence of GM material in other products. These are all highly interdisciplinary and complex issues. A crucial component for a proper assessment is defining the appropriate baseline for comparison and decision. For GM crops, the best and most appropriately defined reference point is the impact of plants developed by traditional breeding, which is an integral and accepted part of agriculture. In many instances, the putative impacts identified for GM crops are very similar to the impacts of new cultivars derived from traditional breeding. When assessing GM crops relative to existing cultivars, the increased knowledge base underpinning the development of GM crops will provide greater confidence in the assurances plant science can give on the risks of releasing such crops.

Where legislation and regulatory institutions are in place, there are elaborate steps to precisely avoid or mitigate these risks. It is the obligation of the technology innovators (i.e., scientists), producers, and the government to assure the public of the safety of the novel foods that they offer as well as their benign effect on the environment. There are also those risks that are neither caused nor preventable by the technology itself. An example of this type of risk is the further widening of the economic gap between developed countries (technology users) versus developing countries (nonusers). These risks, however, can be managed by developing technologies tailor made for the needs of the poor and by instituting measures so that the poor will have access to the new technologies.

Environmental biosafety evaluation

Safety is a relative concept. Agriculture and animal husbandry have inherent dangers, as do the consumption of their products. Any sound evaluation of the safety of genetic engineering must also consider the "safety" of current methods of producing food. Risk is an integral part of everyday life. No activity is without risk. In some cases inaction also entails risk. Agriculture in any form poses risks to farmers, consumers and the environment. Risk analysis consists of three steps: risk assessment, risk management and risk communication. Risk assessment evaluates and compares the scientific evidence regarding the risks associated with alternative activities. Risk management –which develops strategies to prevent and

control risks within acceptable limits– relies on risk assessment and takes into consideration various factors such as social values and economics. Risk communication involves an ongoing dialogue between regulators and the public about risk and options to manage risk so that appropriate decisions can be made (FAO, 2004).

There is broad consensus that the environmental impacts of transgenic crops and other living modified organisms should be evaluated using science-based risk assessment procedures on a case-by-case basis depending on the particular species, trait and agro-ecosystem. The environmental release of transgenic organisms should be compared with other agricultural practices and technology options (FAO, 2004, Ammann *et al*., 2003). All GM crops are thoroughly evaluated for environmental effects before entering the marketplace. They are assessed by many stakeholders in accordance with principles developed by environmental experts around the world. The evaluation procedures include information about the role of the introduced gene, and the effect that it brings into the recipient plant. Also there are specific questions about unintentional effects such as: impact in non-target organisms, whether the modified crop might persist in the environment longer than usual or invade new habitats, likelihood and consequences of the gene being transferred unintentionally to other species (FAO, 2004). A safety assessment is specific to the product and region and considers the nature of the trait, crop plant biology, farming practices and the ecological community among other aspects. Environmental risk assessment and management include: identification of the possible adverse effect, evaluation of the risk magnitude and its possible occurrence, risk characterization, probability of occurrence, consequences of possible adverse effects and design of strategies for risk management

Regulators in different countries typically require similar types of data for environmental impact assessments, but they differ in their interpretation of these data and of what constitutes an environmental risk or harm. Scientists also differ on what the appropriate basis for comparison should be: with current agricultural systems and/or baseline ecological data, in the value of small-scale laboratory and field trials and their extrapolation to large-scale effects and in the use of modelling approaches that incorporate data from geographical information systems. More research is needed on the post release effects of transgenic crops, as well as more targeted post-release and better methodologies for monitoring (ICSU 2003; FAO, 2004). Risk is often defined as “the probability of harm”. A hazard, by contrast, is anything that might conceivably go wrong. A hazard does not in itself constitute a risk. Thus assessing risk involves answering the following three questions: What might go wrong? How likely is it to happen? What are the consequences? (Risk = hazard × probability × consequences). The seemingly simple concept of risk assessment is in fact quite complex and relies on judgment in addition to science. Risk can be underestimated if some hazards are not identified and properly characterized, if the probability of the hazard occurring is greater than expected or if its consequences are more severe than expected. The probability associated with a hazard also depends, in part, on the management strategy used to control it. People are more likely to

accept the risks associated with familiar and freely chosen activities, even if the risks are large. In risk analysis, the following questions should be kept in mind: Who bears the risk and who stands to benefit? Who evaluates the harm? Who decides what risks are acceptable?

Biotech crops contribute to reducing the environmental impact of productive agriculture, thereby increasing global food security without the need for increased land clearance. Insect resistant crops offer an alternative to chemical inputs on some crops and have allowed development of more targeted, flexible, effective and sustainable integrated pest management programmes. Biotech applications in the R&D pipeline (disease resistant, drought and stress tolerant crops, biofortified) offer additional opportunities to increase global food security while further reducing the environmental footprint of agriculture. All possible paths of action must be compared, including inaction, in respect of improving, in a cost-effective and environmentally sustainable way, human health, nutrition, and the ability to afford an adequate diet. The improvement of agriculture and food security depends on several factors. These include stable political environments, appropriate infrastructures, fair international and national agricultural policies, access to land and water, and improved crop varieties, which are suited to local conditions. In particular cases, GM crops can contribute to substantial progress in improving agriculture, in parallel to the (usually slow) changes at the socio-political level. GM crops have demonstrated the potential to reduce environmental degradation and to address specific health, ecological and agricultural problems which have proved less responsive to the standard tools of plant breeding and organic or conventional agricultural practices.

Evaluations that involve both molecular geneticists and environmentalists could help to identify likely applications of genetic technology and establish the most effective and appropriate methods of using them. Considerable experience exists in the introduction of organisms into the environment and this experience can provide some meaningful guideposts. It is also apparent that new genetic tools will open up new opportunities to obtain additional useful knowledge concerning the relationship among various species in the environment. Such knowledge can provide a rational base to the establishment of prudent procedures for future applications. Science cannot declare any technology completely risk free. GM crops can reduce some environmental risks associated with conventional agriculture, but will also introduce new challenges that must be addressed. Society will have to decide when and where genetic engineering is safe enough. As the world population explodes there is an ever-increasing pressure on the earth's limited resources. Thus, it is appropriate that any intervention into the environment be scrutinized carefully. It is also important in such deliberations to consider the alternatives and to measure the `hidden' cost of inappropriately restrictive regulations, the cost of saying 'No'.

Some regulatory requirements can now be modified to reduce costs and uncertainty without compromising safety. Long accepted plant breeding methods for incorporating new diversity into crop varieties, experience from two decades on research on and

commercialization of transgenic crops, and expanding knowledge of plant genome structure and dynamics all indicate that if a gene or a trait is safe, the genetic engineering process itself presents little potential for unexpected consequences that would not be identified or eliminated in the variety development process before commercialization. As in conventional breeding, regulatory emphasis should be on phenotypic rather than genomic characteristics once a gene or trait has been shown to be safe (Bradford *et al.*, 2005).

Introduction to ecology: basic concepts - definitions

What is ecology?

The word ecology, coined in 1866 by the German biologist Ernst Haeckel, derives from the Greek word “oikos” *oikos* meaning "house" or "dwelling", and *logos* meaning "science" or "study". Thus, ecology is the "study of the household of nature", namely the systematic study of the distribution and abundance of living organisms - plants, animals, micro-organisms- and the interactions with one another and with their natural environment. The environment consists of both a living component, the biotic environment (organisms) and a non-living component, the abiotic environment, e.g. physical factors such as temperature, sunlight, soil, rainfall, winds, and marine streams.

Few fields of study are more relevant to the human condition than the field of ecology. The increasing globalization of our economy and social and political structures has resulted in both intentional and accidental introductions of organisms, including pest and diseases, to all corners of the earth – ecological globalization on a grand scale. All the activities of the human populations affect the natural systems. Ecology today, involves several aspects and concerns: a- Interactions organisms-environment; b- Understanding, conservation, restoration and sustainable use of biodiversity; c- Impact of foreign species in ecosystems and d- Strategies for management and reduction of impacts caused by human activity. Critical considerations for ecological studies are that the natural world is diverse, complex and interconnected; that it is dynamic but also stable and self-replenishing; it is organized by physical and biological processes and that the order of nature is affected by human activity.

Life depends upon the physical world, and also affects it. The organisms must continually exchange materials and energy with the physical environment. Organisms interact with one another, directly or indirectly, through feeding relationships, or trophic interactions. Trophic interactions involve biochemical transformations of energy and the transfer of energy from one individual to the next through the process of consumption. Materials move within ecosystems, and the path ways of such movements are closely associated with the flow of energy (Purves *et al.*, 2004). The flow of energy and its transfer efficiency summarize certain aspects of the structure of an ecosystem: the number of trophic levels, the relative importance of detritus, herbivore, and predatory feeding, the steady-state values for biomass and accumulated detritus, and the turnover rates of organic matter in the community. Unlike

energy, nutrients are retained within the ecosystem and are cycled between its abiotic and biotic components.

Human activities modify the great natural biogeochemical cycles and create cycles of synthetic chemicals –pesticides-. These changes can be large enough to cause serious environmental problems. However, ecosystems have the capacity to recover from many disturbances if the alterations have not been too great and the disturbing forces are reduced or eliminated. Controlling our manipulations of biochemical cycles so that ecosystems can continue to provide the goods and services upon which humanity depends is one of the major challenges facing modern societies (Purves et al., 2004).

Organisation of life: hierarchy of interactions - levels of ecological organization

Individual	It refers to the organism inhabiting the environment as an isolated entity or as a member of a social group.
Species	Is the basic lower unit of classification of closely related similar organisms that have a high level of genetic similarity, are capable of interbreeding freely, and are reproductively isolated from other groups. This definition works well with animals. However, in some plant species fertile crossings can take place among related species.
Population	Group of individuals of the same species living in a particular area. Populations are characterized by several parameters, such as abundance and distribution. The amount of resources available, disease, competition for the limiting resources. Predation, birth and death rates, immigration and emigration affect the size of a population. Populations have age structures and age distributions. They are also characterized by an intrinsic rate of increase, the biotic potential (r). Populations do not have unlimited growth, they are limited by the carrying capacity (K) of their habitat. Density dependent and density independent factors also serve to limit growth.
Community	It is made up of the interacting coexisting populations of different species occupying the same geographical area. Communities are characterized by the numbers of species present, their relative abundance, and their feeding and other ecological relationships. Within the community, there is competition for resources, symbiotic relationships may occur and exchange of genes occurs. Populations and communities include only biotic factors

Ecosystem	It is the complex of a living community (biotic factors) and its abiotic factors (soil, rain, temperatures, etc) in a given area. Ecosystems are further influenced by global phenomena such as climate patterns, nutrient cycles, winds. The communities influence the environment, and the environment influences the community, leading to changes and succession in the ecosystem. Energy flow, geochemical, water and nutrient cycling characterize ecosystems. The flow of energy leads to clearly defined trophic structure, biotic diversity, and material cycles (ie: exchange of materials between living and nonliving parts) within the system is an ecosystem.
Biosphere	The totality of ecosystems constitutes the biosphere, the portion of the earth that contains living species. It includes the atmosphere, oceans, soils and the physical and biological cycles that affect them
Biome	Is another level of interaction placed between the ecosystem and the biosphere. It is a major ecological community or complex of communities, extending over a climatically and geographically defined area. There are two broad categories of biomes: aquatic and terrestrial. Biomes are defined based on factors such as plant structures (such as trees, shrubs, and grasses), leaf types (such as broadleaf and needleleaf), plant spacing (forest, woodland, savanna), and climate. Biomes are often given local names. For example, a Temperate grassland or shrubland biome is known commonly as steppe in central Asia, prairie in North America, and pampas in South America.

Attributes of individuals

The ecology of the individual is mainly concerned with the effects of the abiotic and biotic environment on survival and reproduction. Any shortcomings in the phenotype or genotype of an individual will have a selective pressure exerted on them, and the individuals mostly affected by the environment will be removed from the population. This, of course, can be extended up to the species level, where such stabilising selection determines the range of species according to their environmental requirements and susceptibilities

Attributes of populations

Populations have characteristics that define them. They have characteristic distributions over space, and they differ in age and size; they can be clumped, randomly or uniformly distributed in their environment. They have growth rates, which define their abundance. The number of individuals in a population depends on the birth and death rates, and the difference between immigration and emigration. The population has a spatial structure, which includes features such as the density, spacing, and movement of individuals, the proportion of individuals in different age classes, genetic variation, and the arrangement and size of areas of suitable habitat, all of which may vary in space and time. Population structure also affects the

dynamics of parasites and their hosts, including human diseases (Purves et al., 2004). The structure of populations changes continually because demographic events – births, deaths, immigration (movement of individuals into the area), and emigration (movement of individuals out of the area) – are common occurrences. The study of birth, death and movement rates that give rise to population dynamics is known as demography. Individuals within a population compete with each other for resources such as space, mating partners and food. A population continues to grow until the habitat carrying capacity is reached. However, density independent factors such as storms, floods, weather conditions, and natural disasters - earthquakes, volcanic eruptions.

Genetic differentiation of populations depends far less on the movement of individuals among populations than on the forces of selection, mutation, and random change (genetic drift). Gene flow is the exchange of genetic information among populations resulting from the movement of individuals. The genetic structure of a population describes the distribution of the variation among individuals and among subpopulations, as well as the way in which organisms manage the consequences of genetic variation by means of mating systems. Genetic variation is important to a population because it is the basis of the population's capacity to respond to environmental change through evolution (Ricklefs & Miller, 1999). Genetic variation is also important to individuals: variation among an individual's progeny may increase the likelihood that at least some of them will be well adapted to particular habitat patches or to changed conditions. Genetic variation is maintained primarily by mutation and by gene flow from other localities in which different genes have a selective advantage.

Attributes of communities

Factors that define populations also define communities. Communities are usually defined by the nature of the interactions among the populations in the association or by the place in which the association occurs. Communities are characterized by unique interrelated properties: structure and function. Structure is related to the number of species, called species richness, the types of species present and their relative abundances, the physical characteristics of the vegetation, and the trophic relationships among the interacting populations in the community. Rates of energy flow, properties of community resilience to perturbation, and productivity are examples of community function. (Ricklefs & Miller, 1999). The species composition of ecological communities changes constantly over time.

Organisms interact with one another in different ways in their community:

- Two organisms may mutually harm one another. This type of interaction –competition- is common when organisms use the same resource. Intraspecific competition is competition among individuals of the same species. Competition among species is referred to as inter-specific competition.

- One organism may benefit itself while harming another, as when individuals of one species eat individuals of another (*i.e.* herbivores). The eater is called a predator or parasite, and the eaten is called prey or host. These interactions are known as predator-prey or parasite-host interactions. Predators act as evolutionary agents by selecting for adaptation to protect against them (toxic hairs and bristles, tough spines, noxious chemicals, and mimicry).
- Mutualist interaction is when both participants benefit. Mutualistic interactions occur between members of different groups of organisms (between plants and prokaryotes, between fungi and protests, between animals and protists, between animals and plants and with others animals). If one participant benefits but the other is unaffected, the interaction is a commensalism. If one participant is harmed but the other is unaffected, the interaction is an amensalism**.**

Attributes of ecosystems

Ecosystems have trophic levels called *energy pyramids* or *food pyramids*. The first trophic level is made up of primary producers that utilise light energy to make food. These are referred to as autotrophs and are mostly plants (but include also bacteria and algae). Since only photosynthetic organisms are able to use light energy to produce food, they have a key position in the ecosystem. Any factor that affects plants has implications on the ecosystem. The second level is made up of primary consumers, which are the herbivores; then the next level up is made up of secondary consumers, the carnivores; then top carnivores and decomposers saprophytes. The feeding relationships ensures transfer of energy from one level to the other, with only about 10% of energy at the preceding level being available for use at the next level.

Biodiversity: genetic, species and ecosystems

Biodiversity

Biodiversity is the variation of life at all levels of biological organization -genes, species, and ecosystems-. At the UN Earth Summit in Rio in 1992, it was defined as: “The variability among living organisms from all sources, including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems”. The three most commonly studied levels of diversity are: ecosystem diversity, species diversity, and genetic diversity. If the gene is the fundamental unit of natural selection, the real biodiversity is genetic diversity. For geneticists, biodiversity is the diversity of genes and organisms. They study processes such as mutations, gene exchanges, and genome dynamics that occur at the DNA level and generate evolution.

In ecological indexes, Alpha diversity refers to diversity within a particular area, community or ecosystem, and is measured by counting the number of taxa within the ecosystem (usually species)

Beta diversity is species diversity between ecosystems; this involves comparing the number of taxa that are unique to each of the ecosystems. Gamma diversity is a measure of the overall diversity for different ecosystems within a region. Cultural or anthropological diversity is also involved when studying the regional diversity. Biodiversity is not static; it is constantly changing. It is not evenly distributed on earth, and tends to be richer in the tropics. It varies with climate, altitude, soils and other physical parameters. Hotspots, regions with many endemic species, are usually in areas with limited human impact, while the heavily populated regions tend to have the lowest number of species.

Values of biodiversity

Biodiversity has paramount importance for the social, cultural and economic development of humankind. Some ecosystem services that benefit society are air quality, climate (both global CO2 sequestration and local), water purification, disease control, biological pest control, pollination and prevention of erosion. It plays a part in regulating the chemistry of our atmosphere and water supply. Biodiversity is directly involved in water purification, recycling nutrients and providing fertile soils. There are a multitude of anthropocentric benefits of biodiversity in the areas of agriculture, science and medicine, industrial materials, ecological services, in leisure, and in cultural, aesthetic and intellectual value. The most direct and important use of biodiversity is as a source of food. Although a large number of plant species are edible, only a small percentage is used intensively in the production of food with significant nutritional value. Likewise only a few of the numerous animal species are used for food. Plant biodiversity is the basis of development and the sustainability of agricultural production systems. A reduction in the genetic diversity of crops represents an increase in vulnerability to new pests and diseases. The economic value of the reservoir of genetic traits present in wild varieties and traditionally grown landraces is extremely important in improving crop performance (The Academies of Sciences, 2007). Important crops, such as the potato and coffee, are often derived from only a few genetic strains. Improvements in crop plants over the last 250 years have been largely due to harnessing the genetic diversity present in wild and domestic crop plants.

Ecosystem, species, and genetic diversity

Besides the great diversity of life on Earth, our planet also contains a rich variety of habitats and ecosystems. Biodiversity is determined by both its biotic components, represented by the living organisms, and its abiotic components, represented by the characteristics of the places where the organisms live. In a strict sense, diversity is a measure of the heterogeneity of a system. This concept, when applied to biological systems, refers to

the biological heterogeneity that is defined as the amount and proportion of the different biological elements contained in a system.

Ecosystem diversity comprises the diversity of natural and artificial habitats, plus the species complexes they contain. Species are assembled in distinct ecological systems, such as a tropical forest, a tropical savanna, or a coral reef. However, measuring ecosystem diversity may be difficult because the boundaries among communities and ecosystems are poorly defined. Human influence on natural ecosystems can result in severe consequences, for example, desertification, poor soils, changes in the atmosphere composition, and pest outbursts.

Species diversity results of the relation between the species richness (number of species) and the relative abundance (number of individuals of each species). A more precise concept is taxonomic diversity, which accounts for the diversity of a group of species more or less related among them. One of the major challenges for today biologists is to describe, classify and give a sustainable use to organisms living in poorly known habitats such as those in tropical rain forests, marine ecosystems and soil communities (Ricklefs & Miller, 1999). Species diversity has an important ecological effect on the structure of communities due to the interactions and interdependences among species: the reduction or disappearance of a given species may influence others that depend on it (WCMC, 2002).

Genetic diversity refers to the variation of genes within any population, among populations or within species. This type of diversity can be characterized at the molecular, population, species or ecosystem level. A lot of attention has been paid to genetic diversity because of its practical applications on plant and animal breeding and production, and on evolutionary studies (Purves *et al.*, 2004).

Megadiversity

The term mega-diversity refers to those areas, which account for a high percentage of the world's biodiversity by virtue of containing the most diverse and the greatest number of plant and animal of species.

Species diversity in natural habitats is higher in warm and rainy zones and decreases as latitude and altitude increase. The richest zones of the world are undoubtedly the tropical rain forests, which cover 7% of the world surface and contain 90% of the insect species of the whole world. (CBD, 2002; WCMC, 2002).

Megadiverse Countries: Brazil, Colombia, Indonesia, China, Mexico, South Africa, Venezuela, Ecuador, United States, Papua-New Guinea, Índia, Austrália, Malaysia, Madagascar, Dem. Rep. Congo, Philippines, Peru

Concentrating on geographical areas and not specific countries, 25 hotspots for biodiversity are identified: Polynesia/Micronesia, Flower Province of California, Central America, Choco/Darien/west Ecuador, central Chile, Caribbean, Atlantic forest of Brazil, Brazilian cerrado, forests of West Africa, the karoo (succulents), the Mediterranean basin,

Madagascar, the coastal forests of the eastern arc of Tanzania and Kenya, the Caucasus, Sri Lanka and the Western Ghats, south-central China, Sundaland, the Philippines, Wallacea, southwest Australia, New Zealand and New Caledonia. These zones occupy only about 1.4% of the earth's surface and contain 44% of the known plants and 35% of the known animals. Tropical forests and Mediterranean zones predominate. Three of the zones are of special importance; Madagascar, the Philippines and Sundaland, followed by the Atlantic forests of Brazil and the Caribbean. The tropical Andes and Mediterranean basin are also important for their rich plant diversity

Problems and threats to biodiversity

Extinction has been a naturally occurring phenomenon over millions of years, without any human involvement. However, due to the human activities and their effect on the environment, species and ecosystems have become increasingly threatened in an alarming way (WCMC, 2002), undermining the basis required for sustainable development. Almost all human activities practiced in a regular basis result in a modification of natural environments. These modifications are harmful to the relative abundance of species and may even lead to their extinction. The main causes of environmental modification are: habitat alteration by i.e. pollutants; habitat fragmentation, which can divide a big population into small subpopulations isolated among each other and increasing their risk of extinction if they are excessively reduced in size; introduction of exotic or non-native species; overexploitation of plants and animals; soil, water and atmosphere pollution; alteration of the global climate; and agroindustries, including forest ones (WRI, 2000). Although the loss of biodiversity in the form of crop varieties and domestic animal races has a small significance if compared to the global biodiversity, their genetic erosion is of immediate concern as it has profound implications and consequences for food supply and sustainability of local practices of animal and agricultural production (WCMC, 2002). Genetic erosion in gene banks is difficult to assess quantitatively. It is usually calculated in an indirect way in terms of cultivated surface with genetically uniform varieties which have selected traits.

After 10,000 years of sedentary agriculture and the discovery of 50,000 varieties of edible plants, only 15 crop species represent today 90% of the food of the world. Rice, wheat and maize are the basic food for two thirds of the world population. The continuous genetic erosion of wild species of cereals and other cultivated plants poses a risk for plant breeding programs. Unless the loss of genetic diversity is controlled, by 2025 about 60,000 plant species —a quarter of the total world capital— might be lost (FNUAP, 2001). Fish stocks are also at risk. FAO estimates that 69% of marine commercial fish supplies of the world have been depleted. The greatest threats to biodiversity are destruction and deterioration of habitats, particularly in tropical developing countries (where biodiversity is concentrated), and introduction of exotic species. Many of the factors affecting biodiversity are related to the needs of agricultural production: the increase in population and the limited arable land, have demanded increased agricultural productivity, and have lead to more intensive agricultural

practices, which has negative impacts on natural biodiversity. Habitat loss due to the expansion of human activities is identified as a main threat to 85% of all species described in the IUCN Red List. Main factors are urbanization and the increase in cultivated land surfaces (Amman et al., 2003).

Causes of biodiversity loss

Biodiversity loss has been indicated by the loss in number of genetic resources and species. It has also been inferred from population decline and the degradation of ecosystem functions and processes. Several causes have been suggested; some of them are direct and some others are identified as underlying factors. Among the direct causes are:

- Habitat conversion/fragmentation
- Unsuitable land use and management
- Domestication/ genetic erosion
- Introduction of invasive and exotic species
- Trade
- Pollution
- Natural events

Among the underlying causes of loss are:

1. Demographic changes
2. Poverty and inequality
3. Climate change
4. Public policies and markets
5. Economic policies and structures.

Climate change is also a factor of biodiversity loss. Excessive burning of fossil fuels is altering the balance of gases in the atmosphere; carbon is building up to levels where the natural ecosystems cannot absorb all of it. For one thing, there are fewer plants to utilize the carbon, and the amounts that are being produced are estimated at 3 billion tons per year. The interdependence of ecosystems is amply demonstrated here. For example, deforestation releases carbon dioxide and methane, which increase global temperatures. It also reduces ground cover, which disrupts the water cycle as well as leading to soil erosion. The soil is washed into lakes and rivers, which silt and reduce aquatic biodiversity among other things.

Although deforestation can be controlled at a local level, the massive amount of deforestation is due to over harvesting of trees for economic use, rather than local use. Most of the fossil fuels generating the carbon in the atmosphere are from the industrialized nations, but the effects are felt throughout the less industrialized world.

Commitments and opportunities

Biodiversity makes part of the national patrimony of each country and represents great environmental, cultural and economic values. Conservation and sustainable use of biodiversity concern all the inhabitants of the world, are an enormous potential for diverse countries and require clearly defined strategies and policies for biodiversity management. As population grows, the demand for freshwater, food and energy resources pushes to risk the sustainability of the environment. Technologies and the way in which we should use them are a growing challenge, and problems related to governability, social organization and human rights are of increasing importance in achieving sustainable results (FNUAP, 2001). In order to feed 8,000 million people that are expected to live on Earth by 2025 and to improve their diets, the world will have to improve food production and work on a more equal distribution of food to avoid malnutrition. Given that the available land surface suitable for agriculture is getting reduced, the increase in production will have to be achieved with higher yields instead of more cultivated surface. For example, scientists are working on genes that help plants to efficiently extract nutrients from soil, which would reduce the need for fertilizers; efforts are directed also to the development of drought resistant plants using the genes through which certain species manage to survive drought. (The Academies of Sciences, 2007). Development strategies that are beginning to materialize in several countries, especially developing ones, are based mainly on a wide use of natural resources in a sustainable way, maximizing the potential of the plant genome sciences toward sustainable and environmentally responsible models of production for food, fuel, and fiber, and incorporating them steadily into the productive sector. Biological resources represent a huge potential, insufficiently exploited, that requires strengthening and applying scientific and technological progress aimed to understand, characterize and use these resources for the benefit of local communities: genomics offers unprecedented tools to use these critical resources (Lemaux 2008).

Evolution and speciation

The Development of Evolutionary Theory

Patterns of reproduction, foraging, social interaction, growth and senescence are shaped by natural selection through the interactions of organisms and their environment. Those behavioral, physiological or developmental responses that allow an organism to accommodate or acclimate to the current conditions are called evolutionary adaptations. In biology, evolution is change in the inherited traits of a population of organisms from one generation to the next. These changes are caused by a combination of three main processes: variation, reproduction, and selection. In a biosafety context, evolution is one of the most important concepts from the point of view of the possible ecological impacts. Some of the principal considerations involve concepts related to natural selection pressures and genotype changes (which affect the rate of evolution), phenotypic variance, heritability, response to selection, and inbreeding – out-crossing and genetic variation among others.

Biology became a defined science when the British Charles Darwin published "On the Origin of Species." Darwin's Theory of Evolution is the widely held notion that all life is related and has descended from a common ancestor. Darwin's general theory presumes that complex creatures evolve from more simplistic ancestors naturally over time. In a nutshell, as random genetic mutations occur within an organism's genetic code, the beneficial mutations are preserved because they aid survival -- a process known as "natural selection." These beneficial mutations are passed on to the next generation. Over time, beneficial mutations accumulate and the result is an entirely different organism (not just a variation of the original, but an entirely different creature). Natural selection acts to preserve and accumulate minor advantageous genetic mutations; is the preservation of a functional advantage that enables a species to compete better in the wild. Natural selection is the naturalistic equivalent to domestic breeding. Over the centuries, human breeders have produced dramatic changes in domestic animal populations by selecting individuals to breed. Breeders eliminate undesirable traits gradually over time. Similarly, natural selection eliminates inferior species gradually over time.

In Darwin's theory of natural selection, new variants arise continually within populations. A small percentage of these variants cause their bearers to produce more offspring than others. These variants thrive and supplant their less productive competitors. The effect of numerous instances of selection would lead to a species being modified over time (Purves *et al.*, 2004). Darwin didn't know that the true mode of inheritance was discovered in his lifetime. Gregor Mendel, in his experiments on hybrid peas, showed that genes from a mother and father do not blend. An offspring from a short and a tall parent may be medium sized; but it carries genes for shortness and tallness. The genes remain distinct and can be passed on to subsequent generations. It was a long time until Mendel's ideas were accepted. Mendel studied discrete traits. These traits did not vary continuously. The discrete genes Mendel discovered would exist at some frequency in natural populations. Biologists wondered how and if these frequencies would change. Many thought that the more common versions of genes would increase in frequency simply because they were already at high frequency.

The evolutionary mechanisms of selection and genetic responses are studied in population genetics, developing quantitative predictions of changes in gene frequencies in response to selection. Hardy and Weinberg showed how genetic variation is retained in Mendelian inheritance, and that the frequency of an allele would not change over time simply due to its being rare or common. Their model (Annex 2) assumed the use of large populations in which there is random mating, no selection, no mutations, and no migration to or from the population. Later, R. A. Fisher showed that Mendel's laws could explain continuous traits if the expression of these traits were due to the action of many genes. After this, geneticists accepted Mendel's Laws as the basic rules of genetics.

Evolution is a change in the gene pool of a population over time. The process of evolution can be summarized in three sentences: Genes mutate; individuals are selected; and populations evolve.

Gene is the unit of genetic inheritance that can be passed on unaltered for many generations. Part of the DNA molecule that encodes a given protein.

Genotype is constituted by all the genetic characteristics that determine the structure and functioning of an organism

Phenotype is the physical expression in the organism of the interaction genotype/environment; the outward appearance of the organism.

Gene pool is the set of all genes in a species or population.

Allele is one of several alternative forms of a gene

Locus is the location of a particular gene on a chromosome

Mutation is a permanent change in the genotype –DNA sequence- of an organism, usually applied to changes in genes to new allelic forms

Recombination refers to the mixing of genetic material via sexual reproduction

Gene flow is the transfer of alleles of genes from one population to another

Genetic basis of the evolutionary mechanisms

Evolution, the change in the gene pool of a population over time; it can occur in different ways. Two mechanisms remove alleles: natural selection and genetic drift. Selection removes deleterious alleles from the gene pool, while drift removes alleles randomly from the gene pool. Three mechanisms add new alleles to the gene pool: mutation, recombination and gene flow. The amount of genetic variation found in a population is the balance between the actions of these mechanisms.

Mutation

Mutations, permanent change in the DNA sequences that make up a gene, range in size from a single DNA building block (DNA base) to a large segment of a chromosome. There are many kinds of mutations. A point mutation is a mutation in which one "letter" of the genetic code is changed to another; lengths of DNA can also be deleted or inserted in a gene. Finally, genes or parts of genes can become inverted or duplicated. Most mutations are thought to be neutral with regards to fitness. Mutations that result in amino acid substitutions can change the shape of a protein, potentially changing or eliminating its function. This can lead to inadequacies in biochemical pathways or interfere with the process of development. Only a very small percentage of mutations are beneficial (Purves *et al.*, 2004). A change in environment can cause previously neutral alleles to have selective values; in the short term evolution can run on "stored" variation and thus is independent of mutation rate.

Recombination

Is the process by which a strand of genetic material is broken and then joined to a different DNA molecule. In eukaryotes recombination commonly occurs during meiosis as chromosomal crossover between paired chromosomes. In general, genetic recombination happens during meiosis, a special type of cell division that occurs during formation of sperm and egg cells and gives them the correct number of chromosomes (haploid). Recombination can occur not only between genes, but within genes as well. Recombination within a gene can form a new allele. Recombination adds new alleles and combinations of alleles to the gene pool.

Gene Flow (migration)

New organisms may enter a population by migration from another population. If they mate within the population, they can bring new alleles to the local gene pool. This is called gene flow. Immigrants may add new alleles to the gene pool of the population, or may change the frequencies of alleles already present if they come from a population with different allele frequencies. It operates when there are no spatial barriers. Gene flow has therefore implications on the introduction of GMOs into an environment, and is therefore the subject of a specific attention in this unit.

Natural Selection

Some types of organisms within a population leave more offspring than others. Over time, the frequency of the more prolific type will increase. The difference in reproductive capability is called natural selection. Natural selection is the only mechanism of adaptive evolution; it is defined as differential reproductive success of pre- existing classes of genetic variants in the gene pool. The most common action of natural selection is to remove unfit variants as they arise via mutation. This is called reproductive success. This is what is commonly referred to as “survival of the fittest”. Fitness is a measure of reproductive success and is due to a number of selection factors:

- Survival/mortality selection. Any trait that promotes survival, increases fitness
- Sexual selection. Sexual selection is natural selection operating on factors that contribute to an organism's mating success. Traits that are a liability to survival can evolve when the sexual attractiveness of a trait outweighs the liability incurred for survival. A male who lives a short time, but produces many offspring is much more successful than a long lived one that produces few.
- Fecundity selection (size of offspring). High fecundity is due to the production of mature offspring due to earlier breeding or number of fertilized eggs produced in species that provide litte or no care for their young. The number of offspring gives family size, e.g. in species that take care of their young

Genetic Drift

Allele frequencies can change randomly. Genetic drift, more precisely termed allelic drift, is the process of change in the gene frequencies of a population due to chance events, which determine which alleles will be carried forward while others disappear. It is distinct from natural selection, a non-random process in which the tendency of alleles to become more or less widespread in a population over time is due to the alleles' effects on adaptive and reproductive success. When sampled from a population, the frequency of alleles differs slightly due to chance alone. Alleles can increase or decrease in frequency due to drift. A small percentage of alleles may continually change frequency in a single direction for several generations. A very few new mutant alleles can drift to fixation in this manner (Purves *et al.*, 2004). Both natural selection and genetic drift decrease genetic variation. If they were the only mechanisms of evolution, populations would eventually become homogeneous and further evolution would be impossible. There are, however, mechanisms that replace variation depleted by selection and drift.

Speciation

Speciation is the evolutionary process by which new biological species arise, in others words speciation is a lineage-splitting event that produces two or more separate species. Many biologists think speciation is key to understanding evolution. There are various types of speciation: allopatric, peripatric, parapatric and sympatric speciation, which differ in geographical distribution of the populations in question. Separate species arose when genetic changes (mutations) between relatives no longer allowed for interbreeding, for instance after geographic separation (Ammann *et al.*, 2003).

Types of speciation (Purves et al., 2004)

Allopatric *(allo=other, patric=place)* is thought to be the most common form of speciation. It occurs when a population is split into two (or more) geographically isolated subdivisions. In order for a speciation even to be considered allopatric, gene flow between the soon-to-be species must be greatly reduced, and eventually the two populations' gene pools change independently until they could not interbreed even if they were brought back together.

Peripatric *(peri=near)* new species are formed in isolated, small peripheral populations which are prevented from exchanging genes with the main population. Genetic drift, and perhaps strong selective pressures, would cause rapid genetic change in the small population.

Parapatric *(para=beside)* the zones of two diverging populations are separate but do overlap; there is no specific extrinsic barrier to gene flow. Individuals mate with their geographic neighbors more than with individuals in a different part of the population’s range. In this mode, divergence may happen because of reduced gene flow within the population and varying selection pressures across the population’s range.

Sympatric (sym=same) occurs when two subpopulations become reproductively isolated without first becoming geographically isolated. Insects that live on a single host plant provide a model for sympatric speciation. If a group of insects switched host plants they would not breed with other members of their species still living on their former host plant. The two subpopulations could diverge and speciate.

The key to speciation is the evolution of genetic differences between the incipient species. For a lineage to split once and for all, the two incipient species must have genetic differences that are expressed in some way that causes mating between them to either not happen or to be unsuccessful. These need not be huge genetic differences. A small change in the timing, location, or rituals of mating could be enough. But still, some difference is necessary. This change might evolve by natural selection or genetic drift. Reduced gene flow probably plays a critical role in speciation. Speciation requires that the two incipient species be unable to produce viable offspring together or that they avoid mating with members of the other group. Some of the barriers to gene flow (reproductive Isolation) that may contribute to speciation are the evolution of different mating location, mating time or mating rituals; the lack of fit between sexual organs or the offspring inviability or sterility. In terms of reproduction, plants have a lot more options than animals do. Many plants can reproduce sexually, by fertilizing other individuals or themselves, and asexually, by creating clones of themselves through vegetative reproduction, while most animals only reproduce sexually. Similarly, in terms of speciation, plants have more options than animals do. Two modes of speciation are particularly common in plants: speciation by hybridization or speciation by ploidy changes (Ricklefs & Miller, 1999).

All species, living and extinct, are believed to be descendants of a single ancestral species that lived more than 3 billion years ago. If speciation were a rare event, the biological world would be very different than it is today. The result of speciation processes operating over billions of years is a world in which life is organized into millions of species, each adapted to live in a particular environment and to use environmental resources in a particular way (Purves *et al.,* 2004).

Extinction

Extinction is a natural process in evolution that occurs when every living individual of a species -or group of taxa- disappear. The history of extinctions on Earth includes several mass extinctions during which large numbers of species have disappeared in a rather short term (Purves *et al*., 2004). The main causes of mass extinctions are disturbances such as: volcanic eruptions, impacts of meteorites, fires, floods, species overexploitation, introducing exotic or non-native species, habitat fragmentation, predation, parasitism, and a reduction of mutualism. Extinction depends on many ecological factors or characteristics of populations (size, geographical distribution, age class structure and spatial distribution). Small populations are in higher danger than large populations, and endemic species —those which are limited to

one or very few populations in specific locations and not found anywhere in the world— are at higher risk than widespread species (Ricklefs & Miller, 1999). The rate of extinction is affected by population size, geographic range, age structure, and spatial arrangement, and may result from a decrease in competitive ability.

Despite mass extinctions, speciation processes (new species arising from preexisting species) have allowed a net increment of species number throughout the history of life on Earth. However, current concern arises due to the accelerated rates of extinction. Scientists calculate that during the past 400 years at least 350 vertebrates and 400 invertebrates have gone extinct and several hundreds of plants have disappeared, as a result of anthropogenic extinction. For 2000, the estimated risk of extinction for mammals was of 24 % and for birds, 12 % (WRI, 2000). Several national and international conservationist agencies have developed strategies and programs aimed at the conservation of wild species. For instance, the IUCN has created the Red Lists of species classifying them into categories according to the level or degree of threat: extinct, extinct in the wild, critical, endangered, vulnerable, susceptible, safe/low risk, insufficiently known and not evaluated. These categories are a guide to conservation activities that must be prioritized.

Agricultural Ecology : Centres of Origin / Diversity

Agricultural activities have become the dominant ecological force over nearly one third of the land areas of the earth. Agro-ecosystems incorporate the concepts of ecology into their design and management. After a long history of separation and lack of interaction, ecologists and agronomists are combining forces to study and help solving the problems confronting our food production a system, facing the natural resources threatens, identifying the ecological problems in agriculture. Application of this knowledge can lead to development of more sustainable agricultural ecosystems in harmony with their larger ecosystem and eco-region (NRCS, 2004). Agro-ecosystems are controlled by definition, by the management of ecological processes.

For 4 million years, people procured food by hunting and gathering. Agriculture began in several places more than 10.000 years ago, and was a necessary condition for the development of civilizations. Crops and farm animals were domesticated and selection took place. Identifying the geographic origin of species is very useful when plant breeders attempt to grow any crop in another zone with different environmental conditions to those of its original zone (Chrispeels & Sadava, 2003). Hybridization has played a major role in the development of new crops, in the modification of existing ones and in the evolution of some troublesome weeds. One of the consequences of agriculture is the conversion of natural ecosystems into crop fields and pastures by removal of climax vegetation, controlling succession and exposing the soil to erosion.

Domestication of species

Domestication is simply accelerated evolution and involves relatively few genes. Domestication implies changes in the genetic makeup and the morphological appearance of plants and behavior of animals, such that they fit the needs of the farmer and consumer. For example, in wheat, as in many other grains, a major difference between the wild progenitor and domesticated descendants lies with seed dispersal. Wild plants spontaneously shed their seeds at maturity in order to assure their dispersal. Early farmers, during domestication have selected plants to hold on their seeds, to minimize yield losses (Chrispeels & Sadava, 2003). The evolution of crops is determined by three bottlenecks for genetic diversity: domestication, dispersal from the domestication centres, and crop improvement in the 20th century. Early agricultural society domesticated few plant species, which were the source of carbohydrates, proteins, fats and fibers. For instance, the emergence of Mediterranean and Middle Eastern civilizations was based on the domestication mainly of wheat, barley, lentils, peas, and linen. Later, the number of domesticated species increased and thus new crops appeared: oat, rye, olives, fruits, and others. Human migrations and exchanges among cultures helped to increase the number of plants cultivated in each region. The discovery of the American continent and all the exchanges that came after led to the greatest levels of genetic diversity within agricultural systems. Unfortunately at the same time, the new available lands began to be used for extensive monocultures especially of coffee, sugarcane, cotton, and tobacco in the colonies of the New World. Agriculture began at similar times in different regions of the world. In each of the regions where the centres of origin are located, human populations domesticated different crops with similar uses.

The domestication of plants and animals is related to the use of a reduced fraction of the existing biodiversity in each region and the adaptation of selected species to new environmental conditions suitable to human use. The domestication by artificial selection to new environmental conditions is opposed to the evolutionary mechanisms of adaptation by natural selection, as the environments where domestication take place differ from the natural environments where wild relatives grow and the selective pressures in each location are different. Domestication results in many morphologic and physiological changes in plants that make them difficult to distinguish from their wild relatives. The most noticeable are related to seed dispersal, seed dormancy, growth type, harvest index, photoperiod, organ size, presence of toxic compounds, and pest and disease resistance. Due to the fact that almost all the crops share the same modified traits that distinguish them from their wild relatives, the whole set of new traits is known as domestication syndrome. Domestication is an artificial selection process directed by farmers. It leads to genetic changes and confers adaptive traits for environmental culture conditions, fitting farmers and consumer's needs.

Centres of origin and diversification

Local and geographic distribution of species depends on ecological conditions, both biotic and abiotic factors, and on evolutionary processes (Purves et al., 2004). The

combination of all of these environmental conditions and processes determines the natural vegetation found in a given region as well as the capability of developing certain crops in particular areas. The geographic distribution of wild relatives of a crop provides a general idea where a crop may have originated. Careful botanic explorations are necessary to determine the precise distribution of wild progenitors. Additional genetic studies involving crosses between the crop and presumptive wild ancestors and a comparison of their DNA can identify in more detail a specific region of domestication.

The Centre of Origin is considered a geographical area where a group of organisms, either domesticated or wild, first developed its distinctive properties. Centres of Origin of cultivated plants are identified on the basis of the number and diversity of wild species as well as the number of endemic species of the concerned genus in a given region, while the Centres of Diversity are recognized on the basis of the number and diversity of different varieties, wild and cultivated, of the species. The Centres of Origin and Centres of Diversity of crop plants as known to us are largely based on circumstantial evidence. In the cases of crops that are extensively cultivated over wide geographical ranges, a large number of new varieties were continuously developed, involving a large number of parents, making the issues virtually intangible. For example, IR-64 rice appears to have had more than 100 parents, with consequent extensive genomic rearrangements, some natural and the others induced (Kameswara & Shantharam. 2004).

Centres of origin and centres of diversification

Centres of origin: The geographic locations where a particular domesticated plant species originated.

These areas are the likeliest sources of natural genetic variation, and represent ideal targets for in situ conservation

Centres of diversity: The locations recognized on the basis of the number and diversity of different varieties, wild and cultivated, of the species.

The most important classification of the centres of origin of cultivated plants was established by the Russian geneticist Nikolai Ivanovich Vavilov (1887-1943). Vavilov realized the importance of genetic diversity of crops and their wild relatives for crop improvement. His most important contribution was the identification of eight major geographic zones, known as “centres of diversity”. There are a limited number of zones where crops originated. They are located in the tropical and subtropical zones, at different elevations, wide variety of topographies, and characterized by distinct dry and wet seasons. They also correspond in many cases to the places where important human civilizations established and flourished.

Centres of origin and domestication of cultivated species

Based on the work of Vavilov in 1949 and Bryant in 2001

I. ***Chinese centre:*** soybean (*Glycine max*), odder radish (*Raphanus sativus*), rapeseed (*Brassica rapa* var. *rapa*), pak-choi (*Brassica chinensis*), Chinese cabbage (*Brassica pekinensis*), Japanese shallot (*Allium fistulosum*), rakkyo (*Allium chinense*), cucumber (*Cucumis sativus*), yam (*Dioscorea batatas*), sorghum, millet.

II. ***Indo Malayan centre****:* Burma and Assam: egg plant (*Solanum melongena*), cucumber (*Cucumis sativus*), mung bean (*Phaseolus aureus*), cowpea (*Vigna sinensis*), taro (*Colocasia esculenta*), yam (*Dioscorea batatas*), rice.

III. ***Indochina and Malayan Archipelago****:* banana (*Musa paradisiaca*), breadfruit (*Artocarpus altilis*), coconut, sugarcane.

IV. ***Indo Afgani-Central Asia centre****:* garden pea (*Pisum sativum*), broad bean (*Vicia faba*), mung bean (*Phaseolus aureus*), leaf mustard (*Brassica juncea*), onion (*Allium cepa*), garlic (*Allium sativum*), spinach (*Spinacia oleracea*), carrot (*Daucus carota* var. *sativus*), apple, chickpea, lentil.

V. ***Near East centre****:* lentil (*Lens culinaris*), lupin (*Lupinus albus*), barley, oat, wheat.

VI. ***Mediterranean centre****:* celery (*Apium graveolens*), asparagus (*Asparagus officinalis*), beetroot (*Beta vulgaris* var. *crassa*), oilseed rape (*Brassica rapa* var. *rapa*), cabbage (*Brassica oleracea* var. *capitata*), parsnip (*Pastinaca sativa*), pea (*Pisum sativum*), rhubarb (*Rheum officinalis*), oat, olive, wheat.

VII. ***Abyssinian centre****:* okra (*Abelmoschus esculentus*), watercress (*Lepidium sativum*), cowpea (*Vigna sinensis*), barley, coffee, sorghum.

VIII. ***Mexico-Central America centre****:* sweet pepper, chili (*Capsicum annuum*), alcayota (*Cucurbita ficifolia*), pumpkin (*Cucurbita moschata*), sweet potato (*Ipomoea batatas*), lima bean (*Phaseolus lunatus*), kidney bean (*Phaseolus vulgaris*), maize (*Zea mays*), tomato.

IX. ***South American centre****:*

X. ***Peru-Ecuador-Bolivia****:* sweet pepper, chili, pumpkin, tomato, kidney bean, potato.

XI. ***Chile****:* potato.

XII. ***Brazil-Paraguay:*** peanut, cassava.

XIII. ***North American centre:*** sunflower.

XIV. ***West African centre:*** millet, sorghum.

North European centre: oat, rye

Agro-ecosystem characteristics

Agricultural ecosystems -agro-ecosystems- have been described as domesticated ecosystems, in many ways intermediate between natural ecosystems (such as grasslands and forests) and fabricated ecosystems –cities (ASAP, 2004). Just as natural ecosystems they can be thought of as including the processes of primary production, consumption, and decomposition interacting with abiotic environmental components and resulting in energy flow and nutrient cycling. Economic, social, and environmental factors must be added to this primary concept because of the human element that is so closely involved with agroecosystem creation and maintenance. Any agroecosystem contains some or all of:

- Crops – plants cultivated for the benefit of human kind
- Weeds – plants that are potential competitors
- Pests - animal predators and parasites
- Pathogens - microorganisms causing diseases
- Domestic animals - animal crops
- Beneficial microorganisms - e.g. rhizobia and other nitrogen fixing bacteria, mycorrhizal fungi
- Beneficial arthropods - pollinators, natural enemies of pests
- Soil

Definitions of agro-ecosystems often include the entire support base of energy and material subsidies, seeds, and chemicals, and even a social-political-economic matrix in which management decisions are made. Agro-ecosystems retain most, if not all, the functional properties of natural ecosystems — nutrient conservation mechanisms, energy storage and use patterns, and regulation of biotic diversity.

Agro-ecosystem patterns and processes

Energy and matter flow in agro-ecosystems is altered greatly by human interference. Inputs are derived primarily from human sources and are often not self-sustaining. They become open systems where considerable energy is directed out of the system at the time of harvest, rather than stored in biomass that could accumulate within the system. In an agro-ecosystem, recycling of nutrients is minimal, and considerable quantities are lost with the harvest or as a result of leaching or erosion, because of a great reduction in permanent biomass levels held within the system. Because of the loss of niche diversity and a reduction in trophic interactions, populations are rarely self-regulating.

Agro-ecosystems are solar powered as are natural systems, but differ from natural systems in that (ASAP, 2004):

- There are auxiliary energy sources that are used to enhance productivity; these sources are processed fuels along with animal and human labor;

- Biodiversity is notably reduced by human management in order to maximize yield of specific foodstuffs (plant or animal);
- Dominant plant and animal species are under artificial rather than natural selection; human inputs determine population sizes -linked to the productivity of the ecosystem.
- Control is external and goal-oriented rather than internal via subsystem feedback as in natural ecosystems.

Creation and maintenance of agro-ecosystems is necessarily concerned with the economic goals of production, productivity, and conservation. They are controlled, by definition, by management of ecological processes and they would not persist but for human intervention. It is for this reason that they are sometimes referred to as artificial systems as opposed to natural systems that do not require intervention to persist through space and time. Knowledge of the ecological interactions occurring within an agro-ecosystem and the sustainable functioning of the system as a whole, allows management for the long term. Sustainability can be achieved in an agriculture that is ecologically sound, resource-conserving and not environmentally degrading.

Sustainable Agriculture

Sustainable agriculture is both a philosophy and a system of farming. It has its roots in a set of values that reflects an awareness of both ecological and social realities. Sustainable agriculture systems are designed to maximize advantage of existing soil nutrient and water cycles, energy flows, and soil organisms for food production. An ecologically sustainable agriculture maintains the resource base upon which it depends, relies on a minimum of artificial inputs from outside the farm system, manages pests through internal regulating mechanisms, and is able to recover form the disturbances caused by cultivation and harvest through successional processes minimizing waste and environmental damage, while maintaining or improving farm profitability (ASAP, 2004). In practice such systems have tended to avoid the use of synthetically compounded fertilizers, pesticides, growth regulators, and livestock feed additives. Natural, biological, and cultural controls are used to manage pests, weeds and diseases.

Management of agro-ecosystems for sustainability both influence and are influenced by biodiversity. Sustainable practices leading to increased crop and genetic diversity have resulted in increased agro-ecosystem stability –increasing crop diversity benefits agriculture by reducing insect pests-. Conservation tillage increases habitat and wildlife diversity, and numbers of beneficial insects species.

Sustainable Agriculture (ASAP, 2004)

- Is based on the prudent use of renewable and/or recyclable resources. It uses renewable energy sources such as biological, geothermal, hydroelectric, solar, or wind.

- Protects the integrity of natural systems so that natural resources are continually regenerated. Sustainable agricultural systems should maintain or improve groundwater and surface water quality and regenerate healthy agricultural soils.
- Improves the quality of life of individuals and communities. In order to stem the rural to urban migration, rural communities must offer people a good standard of living including diverse employment opportunities, health care, education, social services and cultural activities.
- Is profitable. Transition to new ways of knowing, doing and being require incentives for all participants.
- Is guided by a land ethic that considers that long- term good of all members of the land community. An agroecosystem should be viewed as a dynamic interdependent community composed of soil, water, air and biotic species, with capacity for self-renewal.

Many of the approaches in conventional agriculture (minimum tillage, chemical banding) would fall into the "efficiency" category. Efforts to substitute safe products and practices (botanical pesticides, bio-control agents, imported manures, rock powders and mechanical weed control) are being used. Despite the reduced negative environmental damage associated with them, they remain problematic. Botanical pesticides also kill benefic organisms, the release of bio-controls does not address the question of why pest outbreaks occurs, dependence on imported fertilizer materials makes the system vulnerable to supply disruptions, and excessive cultivation to control weeds is detrimental to the soil. As in conventional agricultural systems, the success of sustainable approaches is very dependent on the skills and attitudes of the producers (ASAP, 2004). What has become increasingly clear to a growing number of agricultural professionals in the last few years is that good agronomy is based on an understanding of ecology. An "agro-ecological" approach is used increasingly by agricultural professionals to analyze the success of sustainable farming systems, and to identify ways of improving the productivity profitability, and resource efficiency of them.

Agricultural Biodiversity

Agricultural biodiversity is a broad term that includes all components of biological diversity of relevance to food and agriculture, and all components of biological diversity that constitute the agricultural ecosystems, also named agro-ecosystems: the variety and variability of animals, plants and micro-organisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agro-ecosystem, its structure and processes (CBD, COP decision V/5, appendix). It includes crops and livestock and their wild relatives, but also many other organisms such as soil fauna, weeds, pests and predators. Agricultural biodiversity is the outcome of the interactions among genetic resources, the environment and the management systems and practices used by farmers. This is the result of both natural selection and human inventive developed over millennia.

Dimensions of agricultural biodiversity

1. *Genetic resources for food and agriculture,* which constitute the units of production in agriculture, and include cultivated and domesticated species, managed wild plants and animals, as well as wild relatives of cultivated and domesticated species

- Plant genetic resources, including crops, wild plants harvested and managed for food, trees on farms, pasture and rangeland species
- Animal genetic resources, including domesticated animals, wild animals hunted for food, wild and farmed fish and other aquatic organisms
- Microbial and fungal genetic resources

2. *Components of biodiversity that support ecosystem services* upon which agriculture is based. These include a diverse range of organisms that contribute, at various scales to, *inter alia*, nutrient cycling, pest and disease regulation, pollination, pollution and sediment regulation, maintenance of the hydrological cycle, erosion control, and climate regulation and carbon sequestration

3. *Abiotic factors*, such as local climatic and chemical factors and the physical structure and functioning of ecosystems, which have a determining effect on agricultural biodiversity

4. *Socio-economic and cultural dimensions*. Agricultural biodiversity is largely shaped and maintained by human activities and management practices, and a large number of people depend on agricultural biodiversity for sustainable livelihoods. These dimensions include traditional and local knowledge of agricultural biodiversity, cultural factors and participatory processes, as well as tourism associated with agricultural landscapes

Biodiversity and agriculture are strongly interrelated because while biodiversity is critical for agriculture, agriculture can also contribute to conservation and sustainable use of biodiversity. Indeed, sustainable agriculture both promotes and is enhanced by biodiversity. Maintenance of this biodiversity is essential for the sustainable production of food and other agricultural products and the benefits these provide to humanity, including food security, nutrition and livelihoods.

Conservation of genetic resources

Genetic Resources for Food and Agriculture

Genetic resources for food and agriculture are the basis of global food security. Genetic resources for food and agriculture are the biological basis of world food security and, directly or indirectly, support the livelihoods of every person on Earth. Used either in traditional farming and breeding, or in genetic engineering, they constitute a world patrimony of invaluable usefulness for humankind existence. Plant genetic resources comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species (FAO 2002). Genetic diversity provides farmers and plant breeders with

options to develop, through selection and breeding, new and more productive crops, resistant to virulent pests and diseases and adapted to changing environments.

Genetic diversity of the majority of modern crops is very limited in comparison with their wild ancestors. This reduction in diversity during crop evolution is not recent, as it began with crop domestication. The development of improved '*elite*' varieties during the twentieth century accelerated the rhythm of genetic erosion, which reduces considerably the opportunities for the world community and the small farmers, who depend in many cases on wild species and natural habitats to subsist. The better performance and higher yield obtained with new varieties led farmers to stop using their local varieties and instead to use high-yielding hybrids and new varieties preferred by consumers (Chrispeels & Sadava, 2003). Domestication, artificial selection and constant manipulation of biological diversity by humankind since 10,000 years ago and overall human activities have converted vast forest extensions, savannas and prairies into production fields and industrial complexes. Human societies in the current context are strongly linked to monocultures, the worst condition from the point of view of diversity as it can be attested by the well known devastating consequences of the Irish famine (caused by a potato disease) and the desertification of Sumer in ancient Mesopotamia due to soil salinization (WRI, 2000). Genetic erosion reduces considerably the opportunities for the world community and the small farmers, who depend in many cases on wild species and natural habitats to subsist (Pullin, 2002).

FAO estimates that since 1900, 75% of crop genetic diversity has been lost. Without a constant contribution of new wild genes, plant geneticists and breeders cannot continue improving basic crops. Plants obtained by means of crop selection must be invigorated each 5 to 15 years in order to provide them with new or better traits such as pest and disease resistance, higher yields, or higher tolerance to droughts and saline soils. The most effective way to achieve it is by mixing commercial varieties with wild ones. Many of the local varieties and wild species that are being lost may contain genes with potential utility to plant breeders and biotechnologists for crop improvement (FAO, 2001; WCMC, 2002).

The growing deterioration of the natural and agricultural environments, and concerns for the loss of biodiversity, has resulted in rapid development of the discipline of conservation biology. The origins of gene resource conservation -and the interest of agriculturalists in the origin of domesticated crops and in the use of wild relatives for breeding programs- can be traced to the 1910's. By 1924 the Russian botanist Vavilov founded the All-Union Institute of Applied Botany and New Crops. The number and size of crop gene banks has continued to grow dramatically ever since.

Conservation and restoration

Conservation biology studies the use and management of the biodiversity present in natural and cultivated ecosystems in order to guarantee their renewal, conservation and productivity, thus providing benefits and opportunities for the present and future generations.

The main approaches used today in conservation biology include conservation strategies for undisturbed natural ecosystems, restoration strategies for disturbed ecosystems and sustainable use strategies for transformed ecosystems, which include agroecosystems, urban ecosystems, dams, gardens and recreation areas among others. (WCMC, 2002; WRI, 2000).

When degradation and decline are extreme, and no preservation is possible, restoration ecology studies how to recover and rehabilitate an ecosystem. Restoration involves species reintroduction, the total or partial replacement of extinct populations with the same or similar species having an ecological, social, cultural or economic value. The most effective way to conserve viable populations is to conserve zones which are large enough to allow species and their habitats to exist. An important concept in wildlife conservation is that of biological corridors, which are strips of land connecting fragmented habitats through which species can move from and to different fragments of their natural habitats. Corridors allow the re-colonization of fragments where populations have disappeared and help to avoid inbreeding or endogamy in subpopulations (Pullin, 2002; Ricklefs & Miller, 1999). There is no global consensus as to what constitutes an important species, but species may be singled out for conservation action if they fall into one or more of the following categories: i) Threatened species, ii) Ecologically important species, iii) Species useful to humans, and iv) Species with non-use value.

In situ and ex situ conservation of plant genetic resources (PGR)

As already mentioned, agro-biodiversity is currently threatened by the progressive loss of plant genetic diversity. This problem has increased agriculture vulnerability and has also impoverished food provision for humans (FAO, 2002; Bryant, 2001). The growing concern on genetic erosion has led to the establishment of germplasm conservation programs worldwide. The effort to save biodiversity is directed at both crops and wild relatives. Wild relatives of crops are critical for increasing and improving agricultural production by providing useful genes for resistance against disease and pests, abiotic stress tolerance (drought, salinity, waterlogging), as well as for improving nutritional qualities. They also provide ecosystem services such as pollination, nutrient recycling and water flow management. The effort to conserve the crop wild relatives is happening at national and global level, as this is believed to be one of the most important ways to improve food security. Countries that are richest in genetic diversity are also the poorest in economic terms.

There are two complementary approaches for conservation of plant genetic resources, namely *in situ* and *ex situ*. *In situ* conservation involves maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields as components of the traditional agricultural systems. *Ex situ* conservation, involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration -stored in centralized banks away from the origin. Approaches to *ex situ* conservation include methods

like seed storage banks, field gene banks, botanical gardens, world heritage sites, research centres and laboratories. DNA and pollen storage also contribute indirectly to *ex situ* conservation of PGR (Rao, 2004). Biodiversity International and the Svalbard Global Seed Vault efforts are directed to genetic resources conservation. As a part of the worldwide work, about 2.5 million accessions are being conserved by 700 seed banks around the world, although there has been limited success when using wild seeds in crop improvement crosses (Bryant, 2001).

In situ conservation of plant genetic resources (PGR)

The aim of ***in situ* conservation** is to protect habitats of target species so that natural evolution processes are assured. It includes establishing protected areas such as national parks, caring for peasant plots containing local varieties, preserving forests to protect medicinal or wild species used by indigenous communities. The vision is for the protected areas to include multiple uses, extractive reserves, and use the systems to preserve rare, endangered and threatened species. In these systems, there is a need for increase ecological related to geographic distribution of target species; population structure, dynamics and genetic variability within and between populations; for Identification of threats to target species in the wild and any mitigation actions and for management of ecosystems, including genetic resources, ecological restoration and species recovery plans. The *in situ* conservation strategies include for natural ecosystems: national natural parks, forests, protected areas, reserves and sanctuaries, and for agrobiodiversity: community and domestic parcels including land races and folk varieties. *In situ* conservation of cultivated species is primarily concerned with the on-farm maintenance of traditional crop varieties (or landraces) and with forage and agroforestry species (Rao, 2004). Active participation by farmers and other users of genetic resources is an important part of *in situ* conservation of cultivated species. Crop resources in landraces are passed from generation to generation of farmers and are subject to different selection pressures to fit specific farming situations.

Ex situ conservation of plant genetic resources (PGR)

Among the various *ex situ* conservation methods (germplasm banks), seed storage is the most convenient for long-term conservation of plant genetic resources. Seeds are dried to low moisture content and stored at subzero temperatures in cold stores or deep freezers. According to FAO, this technique accounts for 90% of the 6 million accessions conserved *ex situ* globally.One of the must important examples is the the Svalbard Global Seed Vault, which is a secure seedbank located on the Norwegian island of Sptisbergen in the remote Arctic. The facility was established to preserve a wide variety of plant seeds from locations worldwide in an underground cavern, and holds duplicate samples, or "spare" copies, of seeds held in genebanks worldwide. The Seed Vault will provide insurance against the loss of seeds in genebanks, as well as a refuge for seeds in the case of large scale regional or global crises.

However, there are a large number of important tropical and sub-tropical plant species which produce recalcitrant seeds that quickly lose viability and do not survive desiccation, hence conventional seed storage strategies are not possible. There are also a number of other important crop species that are sterile or do not easily produce seeds, or seed is highly heterozygous and clonal propagation is preferred to conserve elite genotypes.

Ex situ conservation requires skills in management of resources, development of infrastructure and facilities to accommodate the collections. It should be considered as a tool to ensure survival of wild populations and other diversity, and should be integrated into in situ conservation. The collections include: i) Whole plant/animal collections; ii) Zoological parks and botanic gardens where species can be kept without threats; iii) Wildlife research facilities; iv) Germplasm collections of wild and domesticated taxa in any form including zygotes, gametes and somatic tissue. Strategies used in *ex situ* conservation include: Seed banks and germplasm banks, reproduction propagation (as in clonal orchards) and re-introduction into the wild.

Biotechnology for characterization, conservation and sustainable use of biodiversity

Humans have manipulated the genetic make-up of plants and animals since agriculture began more than 10,000 years ago. This exploitation of the natural variation in biological organisms has given us the crops, plantation trees, farm animals and farmed fish of today, which often differ radically from their early ancestors. Increasing the efficiency of agricultural production can reduce these impacts; biotechnologies can have an important role in this respect. Biotechnology is an important tool for biodiversity conservation and utilization, and is a complement – not a substitute – for many areas of conventional agricultural research. It offers a range of tools to improve our understanding and management of genetic resources for food and agriculture. Modern biotechnologies can help to counteract trends of genetic erosion in all food and agriculture sectors (FAO, 2004). One of the most valuable molecular biology techniques are molecular markers, which are used in identification and characterization of species, populations and genotypes, and are very useful for quantifying the genetic diversity within populations. Molecular marker assisted selection (MAS) is a powerful tool in conventional plant breeding and crop improvement programs, because it facilitates the identification of genes with agronomic importance (pest and disease resistance genes), hybridization ratios, to distinguish variety lines, and enables the purity control and certification of varieties(Henry, 2000). Molecular techniques are also useful tools when studying the influence of plant genetic diversity on ecosystem sustainability, due to the fact that diversity within species may contribute in a significant way to the productivity of an agroecosystem. Modern agricultural biotechnology includes a range of tools that scientists employ to understand and manipulate the genetic make-up of organisms for use in the production or processing of agricultural products. Addressing problems such as diseases and pests, abiotic stresses –drought, salinity-, improving nutritional quality, creation of new

diagnosis tools, measurement, conservation and study of genetic resources, production of vaccines, (FAO, 2004).

Germplasm characterization requires observation, measurement and documentation of heritable plant traits. There is need for identification, classification and confirmation of collections by using descriptors:

- *Morphological descriptors*, which are easy, reliable to use and are cheap, but are limited because of limited polymorphisms that can be visualized. They are also affected by the environment, which affects phenotypic expression. These descriptors can also be highly subjective.
- *Agronomic descriptors/traits*, which are useful for crops, but require large-scale field experiments, and are labour intensive.
- *Molecular descriptors*, which use molecular marker technology to identify polymorphisms. These descriptors have proved to be very useful in accessing genes of interest for use in plant breeding and genetic engineering. There is high throughput of information and most techniques are highly repeatable

Biotechnology is being utilized for collecting and storing genes through seed and tissue culture. It is also being used for detection and elimination of diseases in gene bank collections. Identification of desired genes using molecular techniques ensures that the genotypes of choice are used for downstream operations. Long-term storage using cryopreservation of tissue culture results in safer and more efficient storage as well as distribution of germplasm. Molecular techniques are used to confirm identities of germplasm when it is taken out of the banks for regeneration in addition to screening the accessions for identification of genes of interest.

The aim of modern breeders is the same as that of early farmers – to produce superior crops or animals. Conventional breeding, relying on the application of classic genetic principles based on the phenotype or physical characteristics of the organism concerned, has been very successful in introducing desirable traits into crop cultivars or livestock breeds from domesticated or wild relatives or mutants. Biotechnology can make the application of conventional breeding methods more efficient (FAO, 2004). Progress of molecular techniques and in vitro culture of plant organs, tissues and cells has been increasing during the past 50 years. Traditional plant breeding combined with improved agricultural practices and modern biotechnology techniques has resulted in higher crop yields (Henry, 2000). Recombinant DNA technology has been an invaluable tool in crop improvement.

Biotechnology techniques

The most significant breakthroughs in agricultural biotechnology are coming from research into the structure of genomes and the genetic mechanisms behind economically important traits. The rapidly progressing discipline of genomics is providing information on

the identity, location, impact and function of genes affecting such traits –knowledge that will increasingly drive the application of biotechnology in all agricultural sectors. The use and organization of this information is bioinformatics. Advances in bioinformatics may allow the prediction of gene function from gene sequence data; form a listing of an organism's genes, it will become possible to build a theoretical framework of its biology. The comparison across organisms of physical and genetic maps and DNA sequences will significantly reduce the time needed to identify and select potentially useful genes (FAO, 2004).

Molecular markers are identifiable DNA sequences, found at specific locations of the genome, associated with the inheritance of a trait or linked gene. They can be used for i) marker-assisted breeding, ii) understanding and conserving genetic resources and iii) genotype verification. These activities are critical for the genetic improvement of crops, forest trees, livestock and fish. Reliable information on the distribution of genetic variation is a prerequisite for sound selection, breeding and conservation programmes. Genetic variation of a species or population can be assessed in the field or by studying molecular and other markers in the laboratory. A combination of the two approaches is required for reliable results.

- ***Marker-assisted breeding:*** Genetic linkage maps can be used to locate and select for genes affecting traits of economic importance. The potential benefits of marker-assisted selection (MAS) are higher for traits controlled by many genes, such as fruit yield, wood quality, disease resistance, milk and meat production, or body fat. Markers can also be used to increase efficiency of introducing new genes from one population to another, *i.e.* when wishing to introduce genes from wild relatives into modern plant varieties. Key genes for disease resistance and tolerance to acid soils have been isolated from barley and rye using these techniques. Studies carried out using these technologies in fish and forest tree species have revealed high levels of genetic variation both among and within populations. Livestock species are characterized by a high degree of genetic variation within populations, whereas crops exhibit a higher degree of variation across species.
- ***Genotype verification:*** Molecular markers have been widely used for identifying genotypes and for genetic "fingerprinting" of organisms, which is used in advanced tree breeding programmes in which the correct identification of clones for large scale propagation is essential. Genotype verification is used intensively in parentage testing of domestic animals and for tracing livestock products in the food chain back to the farm and animal of origin.

The molecular method used for characterization/conservation depends on the information required:

- If phylogenetic information is required, e.g. to determine origin and relationships of plants, RFLPs and DNA sequencing are the methods of choice.
- If information on **population genetics** is required, e.g. to compare genetic variation, then micro-satellites, AFLPs, RAPDs and allozyme analyses are used.

Cell and tissue culture and micropropagation: The maintenance of genetic collections is a basic requisite for crop improvement. Cryopreservation, an ultra-low temperature conservation technique that uses liquid nitrogen (-196°C) is the only procedure available at present for long-term and low-cost ex-situ conservation of genetic resources. It is widely used for plant species with recalcitrant seeds or vegetative propagation (Engelmann & Takagi, 2000). Micropropagation involves taking small sections of plant tissue, or entire structures such as buds, and culturing them under artificial conditions to regenerate complete plants. It is particularly useful for maintaining valuable plants, breeding otherwise difficult-to-breed species, speeding up plant breeding and providing abundant plant material for research. For crop, horticultural and forest species, micropropagation is now the basis of a large commercial industry involving hundreds of laboratories around the world. Micropropagation can also be used to generate disease-free planting material, especially if combined with the use of disease-detection diagnostic kits.

Genetic transformation: When one desired trait is found in an organism that is not sexually compatible with the host, it may be transferred using genetic engineering. In plants, the most common method for genetic engineering uses the soil bacterium *Agrobacterium tumefaciens* as a vector. Researchers insert the desired gene or genes into the bacterium and then infect the host plant. The desired genes are transmitted to the host along with the infection. Once the gene has been transferred, the crop must be tested to ensure that the gene is expressed properly and is stable over several generations of breeding. This screening can usually be performed more efficiently than for conventional crosses because the nature of the gene is known, molecular methods are available to determine its localization in the genome and fewer genetic changes are involved. Some transgenic crops and traits of greater potential interest for developing countries have been developed but have not yet been released commercially. Nutritionally enhanced crops could make a significant contribution to the reduction of micronutrient malnutrition in developing countries. The well-known transgenic Golden Rice contains three foreign genes – two from the daffodil and one from a bacterium – that produce provitamin A. Scientists are well on their way to developing transgenic "nutritionally optimized"' rice that would contain additionally to the genes producing provitamin A, trhe ones responsible for iron and more protein (Potrykus, 2003). Other nutritionally enhanced foods are under development, such as oils with reduced levels of undesirable fatty acids. In addition, foods that are commonly allergenic (shrimp, peanuts, soybean, rice, etc.) are being modified to contain lower levels of allergenic compounds. Traits for which genetic modification has been contemplated for forest trees include insect and virus resistance, herbicide tolerance and lignin content.

Diagnostics and epidemiology: Plant and animal diseases are difficult to diagnose because the signs may be misleading or even entirely absent until serious damage has occurred. Advanced biotechnology-based diagnostic tests make it possible to identify disease-causing agents and to monitor the impact of disease control programmes to a degree of precision not

previously possible. Molecular epidemiology characterizes pathogens (viruses, bacteria, parasites and fungi) by nucleotide sequencing, which enables their origin to be traced. Enzyme-linked immunosorbent assay (ELISA) tests have become the standard methodology for the diagnosis and surveillance of many animal and fish diseases worldwide, and the polymerase chain reaction (PCR) technique is especially useful in diagnosing plant diseases and is proving increasingly so also for livestock and fish diseases.

DNA based assays are able to detect genetic diversity at higher levels, and unlike morphological descriptors, they are not affected by the environment. Beyond genetic characterization, biodiversity management involves:

- Collection of information to address key issues of both *ex situ* and *in situ* germplasm management. For *ex situ*, molecular techniques are used for sampling, management and development of core collections as well as utilization of genetic diversity. For *in situ*, for identifying the most representative populations within the gene pool of a landrace, considering as well the gene flows occurring in these populations. More than 60% of all *ex situ* accessions in gene banks are for just five species: wheat, barley, rice, maize and beans
- Assistance in decision-making. Out of the whole genome only about 5% is used for coding information and the remainder serves for regulatory control or structural integrity of the genome. Most of these non-coding regions are used to identify:
  - genetic fingerprinting to clarify relationships of taxa. They have been used for crop identification to prove breeders rights and also to identify endangered species in illegal trade,
  - lineages of high conservation, and
  - variation in genotypes.

Genomes contain microsatellites or simple sequence repeats (SSRs) that differ in length and number of repeats among individuals in a population and these repeats are unique for individuals. These characteristics are useful for identification of accessions.

Biotechnology, biodiversity and sustainable agriculture

Biotechnology has the potential to improve sustainability in several ways and is expected, thereby, to help maintain natural as well as agricultural biodiversity. Agriculture has to respond, additional to the traditional focus on higher yields, addressing the protection of environmental goods, consumer concerns for food safety and quality. Biotechnology can overcome some production constraints difficult or intractable by conventional methods. It can speed conventional breeding programmes, create crops resistant to diseases and pests, reducing the use of toxic chemicals that harm environment and human health, and it can provide diagnostic tools and vaccines that help control devastating animal diseases. It can

improve the nutritional quality of staple foods such as rice and cassava and create new products for health and industrial uses (FAO, 2004).

Developing sustainable agricultural systems with minimal impact on biodiversity will require utilizing all available technologies while simultaneously encouraging appropriate farmer practices. Biotechnology should be part of integrated and comprehensive agricultural research and development programmes that gives priority to the problems of the poor. Biotechnology is not a substitute for research in other areas such as plant breeding, integrated pest and nutrient management and livestock breeding, feeding and management systems (FAO, 2004). A great deal needs to be done so that developing-country producers are empowered to make their own decisions regarding these technologies for their own benefit. Identifying small farmers' constraints to technology access and use continues to be an issue that the development community must address. Investments in biotechnology research capacity for the public sector will only be worthwhile if the current difficulties in delivering conventional technologies to subsistence farmers can be reversed (FAO, 2004). We need a better understanding of the sustainability of crop and animal production systems, as well as promoting the development of integrated crop management systems linked to biotechnology progress, in order to establish production systems more friendly to the environment and thus to guarantee resources to future generations.

Gene flow

Gene flow, also known as gene transfer, is the movement or exchange of genes between different species or between different populations of the same species (adjacent conspecifics). Genes may flow (transfer) from one organism to sexually compatible relatives, in which case it is called vertical gene transfer, or by other means –*i.e.* infection- to totally unrelated species and families of organisms, generally referred to as horizontal gene transfer. Gene flow is a natural process, important in the maintenance of genetic variation in populations, as well as in the spread of new traits among populations and across species boundaries, adding new alleles to the gene pool of the populations, or changing the frequencies of alleles present (Ammann *et al.*, 2003). Gene transfer within species is almost essential to preserve the fitness of most species of plants and animals and many species of crop plants and is the basis for evolution. In crops, gene flow typically involves movement of pollen and is dependent upon wind or animal vectors (pollinators). Gene flow occurs with all species, and thus with all crop species, but the amount of gene flow is a function of crop biology. Given its importance, the processes that affect gene flow have been widely studied and generally are well understood.

Vertical Gene Transfer (VGT)

Vertical gene flow occurs naturally between crops and weeds and from crop-to-crop. It occurs between sexually compatible plants and wild relatives if the appropriate conditions are met. Gene transfer between crops and sexually compatible relatives has occurred since the

domestication of plants began more than 10000 years ago. Over the centuries farmers kept seed from the best plants in their crops that had been formed by mutation or had arisen from natural crosses. Gradually, major differences arose between the domesticated and wild species, so that farmers were keeping plants that contained combinations of genes that improved the domestic attributes of the crops (Ammann *et al*., 2003). Most ecological scientists agree that gene flow is not an environmental problem unless it leads to undesirable consequences. In nature gene flow is through pollen transfer to the ovaries. For plants, gene flow may occur in nature by pollen spreading from one population to another. The pollen may be spread in a variety of ways, e.g. by wind, water or insects and other animals. In self-pollinated plants, pollen transfer can be by gravity. Genes from the resulting offspring can be spread further by pollen or by seeds. The minimum requirements for GM gene flow to occur are thus the presence of a sexually-compatible non-GM population in close proximity to the GM population, the possibility of outcrossing between the two populations and the production of fertile hybrids. The degree of outcrossing varies amongst species: e.g. maize and millet are typically cross-pollinated while rice, wheat and barley are primarily self-pollinated. Important aspect is that gene flow refers to the exchange of genes among populations and not simply to the dispersal of pollen or seeds. Introgression is what defines the stable incorporation of genes from one pool to another, and determines the real gene flow between populations.

Since transfer can happen between crops where GM crops are being introduced, it is important to know the crop progenitors as well as their wild relatives in order to assess the likelihood of gene transfer. There is a likelihood of transgene increasing in frequency following gene flow or establishment of feral populations. The answer to the concern of "Does it occur?" now seems clear: gene flow is inevitable from those crops that naturally outcross both to conventional varieties of the same crop and to a small number of wild relatives, although this latter phenomenon is usually a rare event. However, for ecologists and agronomists the key question is "Does it matter?" More specifically, does outcrossing of transgenes affect fitness of recipient offspring in both natural and agricultural ecosystems? The inherent characteristics of a crop and its proximity to closely related plants are some of the factors that determine the likelihood of gene transfer to other plants. The key to understanding gene flow is knowledge of the sexual compatibility of the crop with other species growing in the same landscape.

Factors affecting vertical gene transfer

Gene escape depends on many ecological and agronomical factors: reproductive biology of the plants, whether or not the crop is allowed to flower, how far its pollen travels, success of fertilization, extent of seed dispersal, seed survival, among others. Even if a gene does "escape", its future may be bleak if it handicaps its new host. The probability of success pollination depends on a great number of interrelated factors, including level of pollen production, rate of self- and cross- fertilization of receptor plant, rate of pollen dispersion,

pollinating agents, spatial distance between donor and recipient population, local density of recipient population, difference in phenology between crop and wild population. There is need to evaluate crop and recipient populations' overlap in space and time; hybridization between different crops; the stable incorporation of the transgene into the population (introgression) depends on the fertility of the hybrid produced; and use of landraces, e.g. in Kenya, farmers frequently cross landraces with the improved varieties; thus crop-to-crop gene flow is already widespread.

A trait with selective advantage and improved fitness has a chance of accumulating in offspring of a population, e.g. if the trait is out-crossed with wild relatives, it has a good chance of accumulating in the wild population, and that trait may be preferentially attained. There must be a benefit associated with the given gene in order for it to persist. If there is, for example by increasing survival or reproduction, it is likely to spread more rapidly through the population. Conversely, if it has a detrimental impact on the fitness of individuals, the rate of gene flow is likely to be reduced and the transgene may eventually be lost.

Key Issues to consider for gene flow in crops

- Sexual compatibility between the plants, presence of wild or domesticated relatives
- Pollen production rate
- Out-cross rate and auto-pollination
- Pollen dispersal rate
- Pollen viability and competitive ability
- Characteristics of the pollinator agents
- Spatial distances between GMOs and recipients
- Environmental factors
- Local density of the population
- Temporal differences in flowering -phenologic isolation-. Synchrony of flowering -timing for pollen shed –anthesis- and receptivity must coincide for the crop and nearby relatives
- The resulting offspring must be viable and fertile

Horizontal gene transfer - HGT

Horizontal gene transfer refers to non-sexual transfer between totally unrelated species and families of organisms. HGT is not new: it has occurred during the history of life in earth. It has been a very important feature in the evolution of species and will continue to be important, but there is no obvious reason why its rate should be enhanced by biotechnology. Horizontal gene transfer is very common for bacteria -where DNA move easily between unrelated bacteria-, not so common between other groups of organisms. HGT is a particularly unusual form of gene transfer in that it is frequent and is frequently an essential component of

the pathogenic relationship between the host and microorganism. Gene transfer from bacteria to plants is a well known natural phenomenon and forms the basis for much plant genetic manipulation. The bacteria concerned, *Agrobacterium* species, have evolved a series of plasmid-borne genes that enable them to attach to exposed cells in wounded plants, transferring genes from the plasmid to apparently random sites within the plant genome (Chrispeels & Sadava, 2003). *Agrobacterium* genes are introduced into plant genomes and this constitutes the basis for *Agrobacterium*-mediated plant genetic transformation. There is no evidence to date that other bacteria have evolved specific methods to transfer genes to plants or animals (Ammann et al., 2003).

Differences and similarities between HGT and transposition

It has been argued that HGT is no different from transposition, a natural process that involves genes moving from one locus to another on a chromosome. The so-called jumping genes, or mobile genetic elements, that are also used as vectors in genetic engineering, that were first discovered in maize.

There are similarities between the two, but there are also major and fundamental differences. *Transposons are endogenous in plants*, whereas *transgene are introduced*. Transposition is a rare event that seldom gives rise to new plants, while transgenic plants are grown in large areas. Both transgenes and transposons can silence genes and activate dormant genes. Both are capable of causing mutations. Activation of the transposase gene in plants is not alien, and normally transposons do not give new information in a plant.

The integration of the transposons is regulated by the plant. There is conflicting evidence about the insertion of transposons, some studies show site-specific insertions, while others show no site preferences. In the case of random insertions, the two are similar. In maize, the frequency of transposition depends on the stage of the plant. Activation and deactivation of genes is controlled by the plant. Transgenes on the other hand are present throughout the development of the plant. Transposons are also known to insert in sequences that have been duplicated before, although it is not clear whether this is a consequence of the jump or a presupposition of insertion.

The integration of transgenes is irreversible, while transposon insertion is reversible, although this reversibility might imply higher risk associated with transposition in terms of side effects because of the mutations they cause

Effects of gene transfer

Gene transfer within species is almost essential to preserve the fitness of most species of animals and many species of crop plants, and is very common among micro-organisms. There is an absolute need for the incoming DNA to be replicated if the genes carried are to be maintained in the new host. If DNA that has entered a cell is to be maintained there is a need

for it to confer a selective advantage on the host, or be very closely linked to a gene of this nature. If it does not, the frequency at which genes are present in populations will remain at the frequency at which the genes are inherited. Thus, although some species are very effective in taking-up DNA from the environment, they remain species because integration of foreign DNA is very infrequent and seldom does such integrated DNA confer a selective advantage on the new host. Other factors that are likely to reduce the frequency with which DNA can be maintained in populations are that the different species can have different regulatory signals controlling the expression of genes, and also gene expression can be affected by different preferences for codon usage (Ammann *et al.*, 2003). In crops, the variable homology of the genomes between related species leads to a wide range of possibilities for the introgression rate of a transgene, or any other gene after the F1 hybrid generation. Meiotic abnormalities caused by the distant relationship between parental genomes decrease rates of introgression into new genotypes, thus the production of initial hybrids does not ensure that transgenes will move into weeds or wild relatives. This may result in higher rates of infertility and decreased rates of seed production. Recombination –important process in the incorporation of foreign DNA- is reduced by the unstable chromosome configuration of hybrids produced by distant relatives (Chrispeels & Sadava, 2003). When crosses between plants result in a stable incorporation of genes from one pool to another, differently composed gene pool, the process is called introgression or introgressive hybridization. It is often difficult to prove with certainty because shared traits may also be result of common ancestors or convergent evolution. The most powerful way to detect introgression is by tracking linked molecular markers.

The consequences of the transfer of transgenes to weeds or wild relatives depend on the nature of the novel gene and the biology and ecology of the recipient plant. Gene flow from GM to wild relatives has two potentially harmful consequences: the evolution of increased invasiveness and persistence, and the likelihood of extinction of wild relatives. The transfer of herbicide tolerance is unlikely to confer any competitive advantages to hybrids outside agricultural areas. On the other side, the transfer of traits such as resistance to particular pests and diseases or stress tolerance could potentially give selective advantages to a given plant (increased fitness). Transgenes related to agricultural practices (herbicide tolerance) won't affect non-agricultural environment. Transgenes that provide fitness enhancing characteristics under natural conditions have the potential to disrupt the balance of established ecosystems. In the case of herbicide tolerance, wild weed species may become super weeds. For insect resistance, wild species may become unpalatable and this would affect non-target invertebrates in field margins if their host plants take up the resistance gene. Crops that are being engineered for attributes such as modified starch, reduced pod shatter; virus resistance, etc, may affect wild relatives and cause ecological imbalances.

Measures to limit gene flow in plants: biological and physical barriers

- Separating distance: where purity of a crop is paramount (transgenics grown in the vicinity of organic crops)
- Barrier crops planted around the transgenic crop and this can capture any pollen drift. Serve to dilute pollen being introduced into the next crop, border rows act as buffers to pollen dispersal.
- Crop isolation zones between the GM crop and non-GM crop neighbour, creating a geographic barrier to ensure purity of non-GM crops.
- Manipulation of flowering time or blocking flowering
- Prevent access of pollinators to the flowers of the transgenic plants:
  - Bag flowering structures to prevent pollen spread by insect vectors, wind, or mechanical transfer, or cover female flowers after pollination to prevent loss or dissemination of GM seed
  - If seed production is not required, remove flower heads before pollen and seed production
- Harvest plant material of experimental interest before sexual maturity
- Locate test plots surrounded by roads or buildings.
- Cleistogamy incorporated into the crop so that flowers remain closed during pollinating e.g. as what happens in wheat and soybeans.
- Hybrid barriers. Interspecific incompatibility at the stigma surface or within the style or post-fertilization barriers that cause seed abortion
- Genetic engineering male sterility so that plant produces infertile anthers
- Seed sterility so that GM produces seed that cannot germinate
- Apomixes, the production of seed without fertilization.

Evaluation of gene transfer

Studies in risk evaluation for gene flow must consider primarily, for each crop in each location, the distinctive characteristics of pollen production, as well as the dispersal and potential out crossing. There are three main types of crops: i) Crops with no sexually compatible wild relatives; ii) Crops with wild relatives but with poor compatibility; but spontaneous hybridisation could still occur, e.g. oilseed rape and wild turnip; iii) Crops with fully compatible wild relatives e.g. sugar beet hybridises readily with wild sea beet. The possible implications of hybridization and introgression between crops and wild plant species are so far unclear because it is difficult to predict how the transgenes will be expressed in a related wild species. The fitness of wild plant species containing introgressed genes from a GM crop will depend on many factors involving both the genes introgressed and the recipient ecosystems. While it is important to determine frequencies of hybridization between crops

and wild relatives, it is more important to determine whether genes will be introgressed into wild populations and establish at levels which will have a significant ecological impact (Eastham & Sweet, 2002). The Information needed to assess potential environmental risks associated with outcrossing from transgenic plants include: biogeographical information of the species involved, reproductive biology of the plant and distribution of sexually compatible relatives, and the impact of the introduced trait, if introgressed into other plant species. Currently there are several useful tools available for evaluation such as geographical information systems GIS, modelling, and data related to geographical origin, and region of cultivation. Considerable information already is available on the biology of all major crops, making it relatively straightforward to characterize the likelihood of gene flow for any given crop using published literature and simple field surveys.

Key questions about crop-wild gene flow

- Does the crop occur near its wild relative and flower at the same time? How far can pollen from the crop travel?
- How easily can crop alleles introgress into wild/weedy populations? Do some crop alleles persist indefinitely?
- What is the baseline fitness of crop-wild hybrids compared to the wild relative? Are there strong interactions? Are later generations more fit than early ones?
- Are transgenic traits associated with fitness benefits and/or fitness costs? Could fitness enhancing traits exacerbate weed problems (spread of herbicide resistance) or harm non-target organisms (pollinators)?
- Considerations related with viability and fertility of the hybrid progeny Are the seeds produced viable? Will the plants be fertile and produce viable seeds?

Overall, the potential impacts of gene flow from GM crops are assessed in two steps: (1) the potential for gene flow to occur (likelihood) between the GM crop and any wild relatives is estimated (the exposure component), and (2) the potential environmental impact of gene flow (the hazard component), if it were to occur, is assessed. Gene flow will be higher from crops possessing characteristics that include high pollen production, an ability to disperse pollen over long distances, pollen production over a long period of time, and/or abundant, out-crossing wild relatives.

The development of effective strategies for the safe use of GM crops will depend on adequate biological and ecological characterization of the systems of interest that can only be achieved through a combination of appropriate field tests conducted in relevant environments and development of appropriate models and monitoring methods (Ammann *et al.*, 1999). The Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants of the National Research Council (NRC-CEI, 2002) found that …"the transgenic

process presents no new categories of risk compared to conventional methods of crop improvement but that specific traits introduced by both approaches can pose unique risks".

Ecology of GM Crops : Environmental Effects

Prior to the advent of genetic engineering, plant breeding was not subject to a great deal of regulation. Seed certification standards ensure the purity and quality of seeds, but little attention has been paid to the possible food safety or environmental impacts of new plant varieties derived from conventional breeding. Conventional plant breeding differs considerably from natural selection. Artificial selection and conventional plant breeding break down the resilience in agroecosystems, thereby creating gene combinations that would rarely survive in nature. Conventional breeding has been responsible for a few cases of negative effects on human health. The concerns associated with genetically transformed crops are equally applicable to conventional crops. Most of the world's major food crops are not native to their major production zones; rather, they originated in a few distinct "centres of origin" and were transferred to new production areas through migration and trade. Highly domesticated plants are grown all over the world and migration outside cultivated areas has only rarely caused a serious problem (FAO, 2004, NRC-CEI, 2002). While there are risks associated with the introduction of any novel organisms into a habitat, the ecology of genetically engineered organisms is exactly the same as the ecology of any other living thing (FAO, 2004). The rules are precisely the same, no matter how the genotype is put together.

Ecological risks of GM crops are considered: persistence -the transgenic plants become serious arable weeds; invasion -the transgenic crops become invasive of natural habitats; gene flow -transfer of introduced genes via pollen (or some other process) to other plant species (such that these then become persistent or invasive); reduction of *in situ* biodiversity; development of pests resistant to GM crops; and effects in non-target organisms. The risks are not currently perceived as being high; transfers of genes resulting from conventional crop breeding into non-crop plants has not created conspicuous problems; nor have traditional crop plants themselves become invasive of natural habitats (FAO, 2004). To date, none of the potential risks has been manifest to any significant extent.

The foremost environmental issue is the presence of sexually cross-compatible relatives, whether domesticated or wild. The wild types may be directly related to a crop as progenitors or they may be indirectly related as neighboring taxa. Domesticated relatives are local, farmer selected cultivars, also called landraces. Both wild and domesticated relatives fulfill important roles as reflections of sociocultural identities, production capital of farmers, and repositories of genetic diversity for plant breeders and farmers alike. An important feature of these domesticated or wild relatives is that they generally cross readily with introduced cultivars. This feature sets the stage for potentially extensive gene flow in domestication centers between transgenic cultivars and their relatives. On the one hand, crops have evolved to increase self-pollination, which would reduce gene flow among crop varieties.

An alternative classification groups the concerns by type of impact:

a) ***Impact on the environment***.

- Persistence of the transgene (better adaptation, invasiveness) or the products of the transgene (cumulative effects).
- Susceptibility of non-target organisms.
- Increased use of agrochemicals.
- Unpredictable expression of the transgene or its instability.

b) ***Impact on agriculture and agricultural production***.

- Development of resistance or tolerance in non-target organisms.
- Development of weeds and superweeds.
- Reduction in nutritive value
- Reduction in number of varieties (increase in susceptibility to pest and diseases) and loss of biodiversity (for preference of GM crops over conventional crops).
- Increased costs of agricultural production
- Lack of capacity for risk evaluation and management
- Ethical aspects, labelling (rights to information).

c) ***Impact due to interactions***

- Genetic contamination through pollen and seed dispersal and horizontal transfer of genes
- Transfer of the transgene to micro-organisms (DNA) or generation of new viruses
- Interaction among different GMOs.

Aims of ecological risk assessment:

- to determine the potential for persistence and spread of transgenic crops in a variety of habitats
- to determine the range of plant species that can cross-pollinate with transgenic crops
- to investigate the ecological performance of hybrid plants produced by such pollination
- to develop protocols that would allow crop breeders to carry out their own ecological risk assessments on new transgenic plants in the future.

The risk assessment studies need to consider the fate of the genetically engineered plants (and their pollen) and the effects of the introduction on the environment (i.e. on subsequent crops in the same fields, on adjacent crops, and in nearby natural habitats), considering:

- Problems concerned with the persistence of the vegetative plant and its propagules in different kinds of environments

- Problems related to the spread of the plant by vegetative growth and by seed in both, arable fields and natural habitats
- Problems involving the risks of lateral spread of the engineered genes, either by pollination of different plant species or by other means

Certain principles guide the safety assessment. First, the safety assessment must be specific to the crop and trait involved, and the region where introduction is going to occur in a case-by-case basis. Because the environmental impact of the product will depend upon local conditions and practices, the environmental safety assessment must consider the nature of local agro-ecosystems and farming practices within these systems. Differences in cropping practices and native flora and fauna must be taken into consideration when identifying potential hazards and prioritizing research needs. Second, it is not possible to demonstrate absolute safety for any technology or activity, all technologies and activities carry some risk. Instead, relative safety compared with alternative technologies is what must be assessed (in this case, typically other pest control practices). A regulator must consider whether the product involves greater risks that comparable technologies. Alternatively, the regulator may compare the net benefit (benefit-risk balance) for the product. Note that this risk-benefit balance will reflect local views on the importance of risk and uncertainty, and thus regulators in different regions may make different decisions based on the same data. The assessment then should consider the relative risks and benefits of the new product relative to current practices, and should include the potentially important ecological impacts of these technologies. For an insect-control product like Bt cotton, current practices typically involve the use of conventional insecticides. For herbicide-tolerant crops, that would be other herbicide regimes. These comparisons must be carried out based on local conditions.

Concerns and potential risks of GMOs to the environment

Persistence of the gene or transgene

This includes existence of volunteer plants, increase in capacity to adapt or invade and cumulative effects of transgenic products. In evaluation of possible impacts of a transgenic plant, one of the fundamental issues is to establish whether the introduced genes (traits) can result in the crop becoming more persistent (weedy) in an agro-ecosystem or more invasive in natural habitats. It is known that the characteristics of a weed are the sum of many different traits and that the addition of a single gene is unlikely to turn a plant into a weed. Special attention should be paid, however, to those crops that already have some weed traits or those in which addition of a gene might increase the competitiveness in agro-ecosystems or their invasiveness in natural ecosystems. For example, crops that have a short history of domestication are closest to this situation as they still have wild genes, conferring competitiveness, that are usually eliminated during selection processes to improve a crop. Those GM crops used to date do not show evidence of having increased in persistence or invasiveness. It is important to consider whether a crop is sown in its centre of origin or

domestication, and the type of environment that is introduced into. For this reason risk must be studied and evaluated on a case-by-case and step-by-step basis.

Gene flow and gene dispersal from transgenic crops

Gene flow and gene dispersal are two separate phenomena and their potential consequences are different. Gene flow refers to exchange of genes (transgenes) among species and results in fertilization, whereas gene dispersal solely refers to movement of pollen. Concerns for gene flow are that there will be genetic pollution of species through creation of 'unnatural' hybrids and that a new super-weed species could be created that would have direct consequences for the environment. If gene dispersal has any effect, it is likely to be short-term, but effects of gene flow could be long-lived. Introduced genes could potentially spread in adjacent populations creating new phenotypes. Investigating this requires insight into ecological impacts of such events, including studies of population sizes, dynamics, spread and development to quantify and predict possible scenarios.

An additional factor in the need to restrict inadvertent gene flow is the possibility of generating feral populations of the crop. Many crops do not survive long off-farm, but under semi-natural conditions seed may remain dormant but viable for long periods and feral populations of the crop might establish themselves. This represents a potential problem among members of the cabbage family where species such as rape have become serious weeds. If weedy species contain herbicide resistance genes, for example, they could pose a particularly serious management problem. If these genes were passed among different species within a genus, or among related genera, hybrid weeds could be created. Similar concerns as have been voiced for herbicide tolerance genes will be heard should genetic use restriction technology (GURT) genes be deployed in crop plants. The major fear in this case is that they could be transferred to non-genetically modified crops of the same or related species. The spread of resistance or tolerance genes to pests and diseases has to be considered in a double sense. There are possibilities for those genes to render related weed species more resistant, but depending on the case, they could represent possibilities for better survival of wild species. In general terms, it is likely that they represent an environmental impact only when a new transgene confers enhanced fitness to a crop or its wild relatives with which it is sexually compatible.

In general, assessing the impacts of introducing new technologies into centers of diversity requires a special degree of care for several reasons. There is widespread consensus among scientists and policy makers that the biological and genetic diversity of these regions needs to be preserved, and may be vulnerable to ecological disturbances. Centers of diversity, and centers of origin for crop species, represent areas where many potentially-impacted wild species may exist, including wild relatives of crop species that may be recipients of gene flow, as well as many non-target species that could be directly or indirectly impacted by changes in agro-ecosystems (Lemaux, 2008).

Susceptibility of non-target organisms

Toxicity to living organisms refers to inadvertent effects caused by GMOs to benign organisms in the environment. This can be the case if a GM crop carries resistances to pests and diseases. The ideal situation is to identify a resistance gene to a pest or disease and introduce it such that its product is expressed solely in the tissues where needed. Only then is it likely only to have an effect on the target organism and not on non-target organisms. To do this is not easy however. There are currently advances in this area and there are commercial cultivars with tissue-specific gene expression. For example, there are numerous maize lines that express toxins from *Bacillus thuringiensis* (Bt) specifically to combat insect infestation, others with increased expression of genes for lysine in the grain, canola that expresses male sterility in the pollen, maize with higher oil content in the grain and others with a changed fatty acid profile and polysaccharide reserve structure.

The most studied examples of genetically manipulated resistance in crops are those employing the Bt delta endotoxins. This soil bacterium is abundant under natural conditions and produces a toxin that is lethal to certain insect pests with specific characteristics. One of the most discussed experiments was that involving Bt toxins and the monarch butterfly in the USA. The results of a laboratory study published in 1999 suggested that Bt maize represented a danger to the monarch larvae that consumed *Asclepias spp*. that were covered in transgenic maize pollen. The study did not determine the ecological consequences of the results and the tests were done under laboratory conditions that did not equate with natural conditions. A publication based on results of the experiments generated global interest and stimulated setting up a cooperative research programme in the same year. The research centred on the effects of the supposedly toxic transgenic maize pollen on monarch larvae feeding on pollen-dusted leaves of their food plant. The authors concluded that although the Bt pollen could be toxic at certain high concentrations, under field conditions there was little risk to the monarch larvae as such high concentrations of pollen would be unlikely to occur in nature. The ideal resistance mechanism for pest control would be one with no unwanted adverse effects on other organisms or the ecosystem. It is necessary to evaluate and predict the possibilities for release of a GM crop and pest control in a niche causing a secondary, more competitive, organism to invade.

Ladybirds are generally considered to be beneficial organisms; many eat aphids that are capable of damaging crops through direct feeding and vectoring viruses. Recent studies on the effect of the Bt toxin Cry1Ab from transgenic maize on the biology of the ladybird *Stethorus punctillium* indicated that the toxin had no effect on its fitness. It was shown that the ladybird lacked the mid-gut receptors for the active toxin to bind to. This research indicates that there is a long way to go before the effects of transgenic crops on non-target organisms are sufficiently well understood.

Unforeseen gene expression and instability of transgenes

This potential risk relates to concern over wide crosses in conventional crop breeding. In conventional breeding techniques it is not possible to determine a priori which genes will be introduced. This means a long process of targeted selection after crossing to remove unwanted genes and traits. With GM crops however, it is known with almost certainty which genes are introduced and it is the subsequent laboratory work that determines which will be expressed and will be stable. In general terms, given that there is ample knowledge of the genes and DNA sequences used in genetic transformation, the number of genes introduced into a GM plant is smaller than in a conventional cross. Technical developments mean that a transgene insertion can be specifically located and its expression quite accurately controlled. What distinguishes this technology from the conventional technology is the improved precision in introduction of a small number of well known genes to make for a much better controlled process. To date there appears to be no evidence for such a phenomenon in the GM crops studied and evaluated.

Weeds

Weeds fall into two major classes, parasitic and non-parasitic. Weed control is a major component of crop management programmes. Biotechnology has been less successfully applied to weed management than to management of other biotic stresses. For non-parasitic weeds, biotechnology has been applied to develop herbicide resistance, an indirect control strategy where the crop is the target of the transgenes and not the weed. Species of two parasitic weed genera, *Striga* and *Orobanche*, represent important weeds of the tropics and Mediterranean areas. They are currently managed through various strategies including manual weeding, rotations, chemical control and biocontrol. Biotechnology has potential to transform crops to allow herbicide application for weed control and to alter gene action controlling the stimuli that trigger germination and development of parasitic weed seed. More knowledge of the host-parasite relationship at the molecular level will allow more environmentally sound management methods to be developed.

Parasitic weeds represent a very specific management challenge. Each plant of *Striga hermontheca*, a major problem of cereal crops in the tropics, is able to release 100,000 seeds into the soil, each of which can remain viable for up to fifteen years. There is variation in resistance of some crops, including sorghum, which appears to be under genetic control. This can be selected for using traditional plant breeding methods, but can probably be enhanced in the future using methods from molecular biology such as marker assisted selection (MAS). For crops including maize, there is no naturally occurring host-plant resistance and the only possibilities of obtaining any, though this has not been done yet, would be to induce it or transfer non-host resistance, which occurs in many grass species. Unfortunately, very little is known about the mechanisms of non-host resistance.

Transforming crops to tolerate contact herbicides would not be effective in managing parasitic weeds as they have already done their damage before they appear above the soil surface. Transforming the crop for application of systemic herbicides, as has been done for non-parasitic weed management, is unlikely to be effective as the crop breaks down the herbicide into harmless chemicals that do not consequently reach the parasite, which is intimately linked with the crop via its roots. Enzymes in the crop that are associated with herbicide uptake could be modified to prevent herbicide binding and promote build-up of the herbicide in the parasite. Glyphosate resistance works in this way and it is termed target-site-resistance. This represents the most feasible form of control and has been effective in controlling *Striga* and *Orobanche* infestations in several crops sprayed with several herbicide formulations. Seed dressings that rely on this mechanism can also be used.

One issue constantly being raised is that of the development of a *superweed* which, created through flow of herbicide tolerance transgenes would become impossible to control using standard herbicides. To date such a weed has not developed, but serious weed problems have arisen through deliberate introduction of new ornamental plants and inadvertent introduction of exotics. Some of these have literally become some of the world's worst weeds and yet have been relatively unnoticed by environmental lobby groups. They represent introductions of entire new genomes, and are not merely the result of (trans) gene flow. Perhaps in the future there will generally be a better understanding of the relative level of risk posed by the flow of ethically contentious genes.

Some questions related to the release of genetically engineered organisms can be answered only with practical experience. Realistic, small-scale field tests are the way to evaluate potential risks from commercial scale uses of genetically engineered organisms. However, these short-term studies are only appropriate to risk assessments on annual crop plants. At the end of a three year study of the population biology of transgenic and non-transgenic annual crop plants, one should be in a position to:

- Provide data on persistence and invasion in natural and arable habitats.
- Show how (and if) genetic engineering alters these parameters.
- Describe pollen spread by insect vectors and by other means.
- Show how (and if) genetic engineering alters the production, spread, or compatibility of pollen.
- Catalogue the wild plants that share insect pollinators with the crop.
- Provide quantitative data on successful cross-pollination between the crop and its wild relatives.
- Provide data on the persistence and invasiveness of any transgenic hybrid plants produced by crossing experiments.
- Potential benefits of GMOs

Agrochemicals reduction

The use of industrial agrochemicals has a substantial bearing on the sustainability of agro-ecological systems. Pesticides have not only had direct negative impacts on the quality of the environment, but have also adversely affected biodiversity through removing beneficial and inoffensive organisms. Interestingly, glyphosate, which several crops have been transformed to tolerate, is much less toxic than some of the herbicides (e.g. atrazine) it replaced. There is concern that GMO use in the field of herbicide resistance will result in increased use of herbicides. Evidence suggests that this has not been the case, but that herbicide use has been reduced at the commercial level. Reduction in pesticides can be obtained by identifying, developing and deploying durable host-plant resistance to pests and diseases. Insect pests (9,000 species), plant pathogens (50,000 species) and weeds (8,000 species) account for the greatest crop losses, and their control requires the greatest use of agrochemical crop protection. The advantages of host-plant resistance are numerous and include: It is relatively inexpensive for the farmer in comparison with chemical control; It is always present.It has no effect on organisms other than the target ones; It can be extremely durable; It can employ a diversity of resistance genes; It does not interfere negatively with other forms of control; It has no negative effects on yield. There are also countless possibilities to improve crop production through breeding for adaptation to a range of abiotic stresses, including drought, salt and heat, and more efficient use of nitrogen and water.

Biotechnology applications to date have focussed on engineered traits such as herbicide resistance for some of the major commodity crops, but there is considerable potential for expanding the methods to include a broader range of crops and genetically more complex traits. Many disease resistances are governed by few genes and represent relatively easy targets for the molecular breeder. Resistance to some diseases is controlled by many genes, each of small effect. Using modern methods, including QTL (Quantitative Trait Loci) analysis, important areas of the genome in resistant lines can be identified, located and ultimately cloned for inclusion into susceptible, but otherwise adapted, germplasm.

GURTs - Genetic use restriction technology

GURTs (genetic use restriction technology) are molecular switches that can be inserted into plants to control seed fertility. GURT technology is very far from being fully developed and can only be used in plants that can be successfully transformed. There are fundamentally two classes.

- V-GURTs are variety use restriction types that work in various ways, but seed sterility results from disrupted embryo formation. A disrupter gene is inserted into the plant that can be induced through chemical seed treatment. Alternatively, the default can be that the seed is rendered sterile through incorporation of a gene that is inactivated during the breeding process by chemical application that restores seed fertility. A similar mechanism is potentially feasible for application in crops that are reproduced vegetatively, whereby

plant growth is inhibited or restored through activation and deactivation of transgenes. This would theoretically extend shelf-life of the crop.

- T-GURTs, which relies on restriction of a specific trait, which is nearer application. T-GURTs rely on a trait being controlled through inducible promoters that silence genes, or through physical gene removal using enzymes

The nature of the impacts of deployment of GURT technology will differ according to country infrastructure and farming system. Intensive agriculture is the norm in developed countries and farmers are used to relying on private industry for a whole range of agricultural inputs, including seed. There is little reliance on the public sector for such inputs and saved seed is not a major feature of the farming systems, although it is still practised.

For very large numbers of farmers in developing countries however, seed is saved from one season to the next to perpetuate the crop. For these farmers GURT technology would certainly restrict their operations were it to be introduced into their crops. The social structure of many farming communities could be disrupted by such a technology as GURT. Germplasm exchange between farmers would almost certainly be limited by GURTs, thereby reducing agro-biodiversity. Many of the consequences of introductions of GURTs might parallel those of Green Revolution technologies, with changes in patterns of land ownership, reduced reliance on the public sector for inputs, a widening gap between resource-rich and resource-poor farmers. There would be serious consequences for developing country agriculture if seed monopolies developed, or worse if companies collapsed and farmers were unable to purchase alternative varieties.

GURTs are ethically contentious, and have potentially negative socio-economic and environmental consequences, particularly for farmers and ecosystems in developing countries. They are not however, without some potential advantages and could conceivably be a useful additional or alternative regulatory tool in specific circumstances. If research and development of GURTs continues, their applications will probably have to be considered on a case-by-case basis.

Potential benefits

One of proposed benefits of GURTs is that they can be used to restrict the spread of transgenes into the environment by rendering sterile any seed produced by accidental transgenes.

Potential risks

There is a potentially very serious consequence for farmers, particularly those in developing countries, of not being able to save seed from GURT-transformed crops for sowing in subsequent seasons. Much in the same way that hybrid seed results in increased reliance of farmers on seed companies, seed of GURT-transformed crops would have to be purchased each season.

The greatest potential threat of GURTs probably comes from the possibility of transgenic pollen from out-crossing crops spreading to neighbouring fields of non-transgenic crops, and the use of chemicals to trigger or switch-off gene expression.

Although GURTs are purported to be useful in restricting potentially deleterious dissemination of transgenes, it is possible that the very molecular switching mechanism that controls seed sterility could enter the ecosystem via release of transgenic pollen or transfer by vectors, such that crops, crop relatives and wild species could be rendered sterile. The same fears over biosafety of transgenic crops in general apply to GURT-transformed species. In addition, inducer chemicals used to inactivate and activate the molecular switches include some, such as steroids and antibiotics, which could harm the environment and human health.

References

ACRE - Advisory Committee on Releases to the Environment UK. 2007. Managing the Footprint of Agriculture: Towards a Comparative Assessment of Risks and Benefits for Novel Agricultural Systems. Report of the ACRE Sub-Group on Wider Issues raised by the Farm-Scale Evaluations of Herbicide Tolerant GM Crops. Available online at www.defra.gov.uk/environment/acre/

AGBIOS - Agriculture & Biotechnology Strategies, Inc. 2002. Essential Biosafety™. A comprehensive source of scientific and regulatory information. CD-ROM^{2nd} Edition.

AGRIFOOD AWARENESS. 2004. New Co-Existence Study on GM and Non-Gm Crops in the EU. http://www.pgeconomics.co.uk/pdf/Co-existencestudyEU_PG_Economicsmay2004.pdf.

AMMANN K., Y. JACOT, V. SIMONSEN & G. KJELLSSON (Eds.). 1999. Methods for Risk Assessment of Transgenic Plants. III. Ecological risks and prospects of transgenic plants, where do we go from here? A dialogue between biotech industry and science. Birkhaüser-Verlag, Basel, Switzerland. 260 p.

AMMANN, K. 2003. Biodiversity and Agricultural Biotechnology: A review of the impact of agricultural biotechnology on biodiversity. Botanischer Garten Bern, Switzerland. 54 p.

AMMANN, K. 2007. Reconciling Traditional Knowledge with Modern Agriculture: A Guide for Building Bridges. In Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices (eds. A Krattiger, RT Mahoney, L Nelsen, et al.). MIHR: Oxford, U.K., and PIPRA: Davis, U.S.A. Available online at www.ipHandbook.org.

AMMANN, K., Y. JACOT & R. BRAUN (Eds.) 2003. Methods for Risk Assessment of Transgenic Plants. IV. Biodiversity and Biotechnology. Birkhaüser-Verlag, Basel, Switzerland 177 p.

ASAP Agroecology/Sustainable Agriculture Program 2004. Principles of Sustainable Agriculture. http://www.aces.uiuc.edu/~asap/index.html

BENBROOK, C.M. 2002. A perspective on actual versus potential environmental benefits of agricultural biotechnology. In: Environmental Savior or Saboteur? Debating the Impacts of Genetic Engineering. February 4, San Francisco, California. http://www.biotech-info.net

BRADFORD, K.J., Van DEYNZE A., GUTTERSON, N., PARROT, W. & STRAUSS, S.H. 2005. Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. Nature Biotechnology **23**: 439- 444.

BROOKES, G. & BARFOOT, P. 2006. Global Impact of Biotech Crops: Socio-Economic and Environmental Effects in the First Ten Years of Commercial Use. AgBioForum **9**(3): 139-151.

BROOKES, G. & BARFOOT, P. 2008. GM crops: global socio-economic and environmental impacts 1996-2006. PG Economics Ltd, UK.

CHRISPEELS, M. J. & D. E. SADAVA. 2003. Plants, Genes and Crop Biotechnology. Second Edition. American Society of Plant Biologists and the ASPB Education Foundation. Jones and Bartlett Publishers, Salisbury, Massachusetts, USA.

CIAT-UN-BIODIVERSITY- REDCAPA-CTA 2007. Multi-Institutional Distance Learning Course on the Ex-situ Conservation of Plant Genetic Resources. Available online at: www.ciat.org

COHEN, J.I. 2005. Poorer nations turn to publicly developed GM crops. Nature Biotechnol. **23**: 27-33

CONNER, A.J., T.R. GLARE & J.P. NAP. 2003. The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. The Plant Journal **33**: 19-46

DALE P.J., B. CLARKE & E.M.G. FONTES. 2002. Potential for the environmental impact of transgenic crops. Nature Biotechnology **20**: 567-574.

DANIELL, H. 2002. Molecular strategies for gene containment in transgenic crops. Nature Biotechnol. **20**: 581-586.

DEFRA - Department for Environment, Food & Rural Affairs UK. 2002a. Guidance on Best Practice in the Design of Genetically Modified Crops. www.defra.gov.uk/environment/acre/bestprac/consult/guidance/

DEFRA - Department for Environment, Food & Rural Affairs UK. 2002b. Guidance on the Assessment of Risk to Wider Biodiversity from Proposed Cultivation of GM Crops. www.defra.gov.uk/environment/consult/biodiversity/risk/index.htm

DEFRA. - Department for Environment, Food & Rural Affairs UK. 2002c. Farm-scale evaluations of genetically modified crops. Answers to some frequently asked questions. www.defra.gov.uk/environment/fse/raq/index.htm

EASTHAM, K. & J. SWEET. 2002. Genetically Modified Organisms (GMOs): The significance of gene flow through pollen transfer. European Environment Agency EEA´s Corner Series Environmental Issue Report Nº 28. 75 p.

EDMEADES, S., & SMALE, M. 2006. A trait-based model of the potential demand for a genetically engineered food crop in a developing economy. Agricultural Economics **35**(3): 351–361.

FAO - FOOD AND AGRICULTURE ORGANIZATION 2001. Genetically modified organisms, consumers, food safety and the environment. Editorial Group FAO Information Division Rome. www.fao.org

FAO - FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2004. The state of food and agriculture 2003-2004. Agricultural Biotechnology: Meeting the needs of the poor? FAO Agriculture Series Nº35.

HALPIN, C., 2005. Gene stacking in transgenic plants – the challenge for 21st century plant biotechnology Plant Biotechnology Journal 3, pp. 141-155

HENRY, R. J. 2000. Practical applications of plant molecular biology. Chapman & Hall, London, UK, 258 p.

HOTA, M. 2007. Crop Biotech & Biosafety. International Service for the Acquisition of Agri-biotech Applications (ISAAA), New Delhi.

ICSU – International Council for Science - Nuffield Council on Bioethics. 2003. GM Science Review Panel. 298 p.

IFPRI – International Food Policy Research Institute. 2008. Insecticide Use on Vegetables in Ghana. Would GM Seed Benefit Farmers? IFPRI Discussion Paper 00785 - Environment and Production Technology Division. Available in: www.ifpri.org

JAMES, C. 2007. Global Status of Commercialized Biotech/GM crops: 2007. ISAAA Brief No. 37. ISAAA: Ithaca, NY.

KAMESWARA RAO, C. & S. SHANTHARAM. 2004. Can GM Crops be introduced into Crop Centres of Origin ad Diversity? Special to AgBioView, April 13, 2004

KOLADY, D. E. & LESSER, W. 2008. Is genetically engineered technology a good alternative to pesticide use? The case of GE eggplant in India. International Journal of Biotechnology: 10 (2/3): 132–147.

LEMAUX, P.G. 2001. Nudging mother nature: the promise and reality of AgBiotech for farmers. http://ucbiotech.org/ resources/biotech/talks/

LEMAUX, P.G. 2008. Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part I). Annu. Rev. Plant Biol. **59**: 771-812

MILLER, H., MORANDINI, P., & AMMANN, K., 2008. Is biotechnology a victim of anti-science bias in scientific journals? Trends in Biotechnology, Electronic Prepublication Febr. 17, 2008, Hardcopy available in March, pp 122-125 Available online at http://www.botanischergarten.ch/Peer-Review/Miller-Morandini-Ammann-Peer-Review-2008.pdf

NAP, J.P., A. ATANASSOV & W.J. STIEKEMA. (Eds.) 2004. Genomics for Biosafety in Plant Biotechnology. NATO Science Series. IOS Press, Amsterdam, The Netherlands. 247 p.

NRC-CEI - NATIONAL RESEARCH COUNCIL COMMITTEE ON ENVIRONMENTAL IMPACTS. 2002. Environmental Effects of Transgenic Plants. National Academy Press, Washington, D.C. 320 p.

NRCS – NATURAL RESOURCES CONSERVATION SERVICE - USDA. 2004. Agricultural Ecosystems and Agricultural Ecology. www.nrcs.usda.gov

PIDGEON, J.D., M.J. MAY & A.M. DEWAR. 2003. GM crop management for environmental benefit. In: 7th ICABR International Conference: Public Goods and Public Policy for Agricultural Biotechnology. Ravello (Italy), June 29 – July 3.

POTRYKUS, I. 2004. Experience from the Humanitarian Golden Rice Project: Extreme Precautionary Regulation Prevents Use of Green Biotechnology in Public Projects. BioVision Alexandria 3-6 April 2004.

PULLIN, A. S. 2002. Conservation Biology. Cambridge University Press. Cambridge, UK. 345 p.

PURVES, W.K.; D. SADAVA; G.H. ORIANS & H.G. HELLER. 2004. Life: The Science of Biology. Sinauer Associates, Inc., Sunderland, Mass., USA. 7th Edition. 1121 p.

RAO, N.K. 2004. Plant genetic resources: Advancing conservation and use through biotechnology African Journal of Biotechnology **3**(2): 136-145

RICKLEFS, R.E. & G. L. MILLER. 1999. Ecology. W. H. Freeman & Co., New York, USA. 4th Edition. 822 p.

RULL, V. 2008. Speciation timing and neotropical biodiversity: the Tertiary–Quaternary debate in the light of molecular phylogenetic evidence. Molecular Ecology **17**: 2722–2729

The National Academies USA. 2007. New Horizons in Plant Sciences for Human Health and the Environment. Available online at: http://www.nationalacademies.org/plant_genome

THE WORLD BANK. 2007. World Development Report 2008. Agriculture for Development. Available online at: www.worldbank.org

TRAYNOR, P.L., FREDERICK R. & KOCH M. 2002. Biosafety and Risk Assessment in Agricultural Biotechnology (A Workbook for Technical Training). The Agricultural Biotechnology Support Project, Institute of International Agriculture, Michigan State University, USA. 142 p.

UN Millennium Project 2005. Investing in Development: A Practical Plan to Achieve the Millennium Development Goals. Overview.

VAVILOV, N.I. 1997. Five Continents. (Rodin LE,Ed.) International Plant Genetic Resources Institute, Rome, Italy. 198 p.

WCMC WORLD CONSERVATION MONITORING CENTRE. 2002. Biodiversity: An Overview. www.wcmc.org.uk/ infoserv/biogen/biogen.html

In: Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues. Chowdhury MKA, Hoque MI and Sonnino A (Eds.). pp. 107-156. © FAO 2009.

Chapter 3: Risk Analysis for GMOs: Concepts, Methods and Issues

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Introduction

Modern biotechnology[1] or genetic engineering has numerous applications in medicine, industry and agriculture. It is well recognized that modern biotechnology has significant potential to contribute to increased agricultural productivity, enhanced food security and national development particularly for developing countries where agriculture is important. Applications of genetic engineering in agriculture have led to the introduction of new traits into plants, animals and micro-organisms and the creation of genetically modified organisms (GMO)[2], which are then used to grow/produce and manufacture GM foods. In 2007, commercial GM crops are grown in 114.3 million hectares in 23 countries with traits introduced primarily to protect the crops from pests and diseases (James, 2007). With increasing adoption, and more diverse products in the pipeline, GMOs are expected to become widespread.

Despite the enormous potential benefits of modern biotechnology, there is considerable international concern that products of modern biotechnology may pose potential risks to human health and the environment. With genetic engineering, it is now possible to manipulate the genetic materials of unrelated species that can result in new gene combinations not previously seen before. This led some to who are concerned with the environment to fear that the resulting GMOs will become invasive, harm other non-target and endangered species, result in loss of biodiversity, etc. From the perspective of human health, some believe that GM foods produced through modern biotechnology are distinctly new and different from conventional foods. Thus, they are potentially dangerous (i.e. toxic) and must be avoided. Beyond these safety considerations, there are also concerns that GMO may result in seed monopoly, are detrimental to farmer's rights to save seeds; and ethically wrong ("playing God"), etc.

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[1] The definition of modern biotechnology from the Cartagena Protocol on Biosafety is adopted: "Modern biotechnology means the application of: *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers that are not techniques used in traditional breeding and selection."

[2] In this document, the term 'genetically modified organism' (GMO) is meant to be the same as the 'living modified organism' (LMO) of the Cartagena Protocol on Biosafety. Other terminologies used for "genetically modified " are "genetically engineered", "transgenic", "bioengineered" and "products of modern biotechnology"

Because of the potential human and environmental risks, real or perceived, posed by GMOs, it is widely recognized that there is a need for each country to establish a regulatory regime specifically to assess the safety of products of modern biotechnology. There are several options available[3] that each country may choose to enable them to explore the benefits of modern biotechnology while at the same time address concerns about potential adverse effects of the introduction of GMOs to human health and the environment. The options are related to the design and objectives of the regulatory system, implementation mechanism and regulatory structures, and other considerations that includes public participation, stand alone or integration into other national objectives and harmonization with other regional and international obligations. Whatever option the country selects, a biosafety framework typically includes four important elements: a national biosafety policy instrument (e.g law, act or decree), a regulatory system, a system for monitoring and compliance and procedures for ensuring transparency, public participation and accountability.

The Cartagena Protocol on Biosafety (CPB), an international multilateral agreement on biosafety, was adopted in 2000 and came into force in 2002. It has been signed by more than 100 countries in recognition of the need to ensure biosafety through national systems of risk assessment. Its main objectives are: to set up the procedures for the safe transboundary movement of living modified organism, harmonize principles and methodology for risk assessment and establish a mechanism for information sharing through the Biosafety Clearing House (BCH). In addition to environmental safety, the Codex Alimentarius Commission has also adopted safety principles and guidelines for risk assessment of GM foods and food products derived from modern biotechnology.

The Government of Bangladesh, through the Bangladesh Department of Environment officially released the 'National Biosafety Framework 2007 (NBF)' along with the revised 'Biosafety Guidelines of Bangladesh 2006 (Biosafety Guidelines) on May 10, 2008. Currently, these are non-statutory instruments that outline the regulatory framework specifically applied to regulate the conduct of activities of modern biotechnology and assess the safety of products to the environmental and human health. As a Party to the Cartagena Protocol on Biosafety (CPB), a new act or law is expected to be promulgated to meet the country's obligation as a Party to the Protocol and harmonize it with other regional and international obligations that address biotechnology-related concerns.

The Bangladesh NBF provides the regulatory framework for biosafety that includes a mechanism for risk analysis and decision-making in regard to authorization and issuance of licenses/permits for all dealings[4] with GMOs. The components of the risk analysis process are: risk assessment, risk management and risk communication. The Biosafety Guidelines

[3] World Bank Agricultural and Rural Development Department Report No. 26028. Biosafety Regulation A Review of International Approaches April 2003

[4] 'Deal with GMOs' means " (a) conduct experiments with GMOs; (b) make, develop, produce or manufacture GMOs; (c) breed GMOs; (d) propagate GMOs; (e) use GMOs in the course of development or manufacture of a thing that is not GMOs; and (f) grow, raise or culture GMOs etc." Definition is adopted from the Bangladesh National Biosafety Framework (Chapter 3.5.3).

contain standards and codes that cover aspects of risk assessment and safety requirements and risk management needed for undertaking (a)Laboratory work, (b) Field trial and (c) Commercial use, involving i) Microorganisms ii) plants and iii) Animals.

Using the Guidelines, the Technical Committee on Crop Biotechnology in the Ministry of Agriculture has approved the import for contained trials of GM crops (Golden Rice, Bt eggplant, late blight resistant potato, Bt chickpea, and ring spot virus resistant Papaya). Bt. eggplant has completed the limited field trials and is waiting for the next stage of approval for multi-locational trials in farmers' fields. However, Bangladesh has not yet allowed commercial propagation and imports of GM food and agricultural products.

This Lecture Module was prepared provide the participants with information on the basic concepts of biological risks, concepts, principles and methodologies of risk assessment, risk management (except monitoring which is addressed in another lecture module) and risk communication. It focuses on crop biotechnology and environmental risk assessment of GM crops since these are the GMOs that are of immediate interest to Bangaladesh. Food safety assessment is not addressed specifically because it is not within the scope of this Training Workshop. This lecture does not cover risk assessment of GM animals and GM micro-organisms.

Biological Risk: Basic Concepts and Classifications

Biological Risk

Biological risk refers to naturally occurring or human made risk caused by exposure to biological agent or microorganism. The following terminologies associated with biological risks are defined or described:

Risk - likelihood that under particular conditions of exposure an intrinsic hazard will represent a threat or harm; risk is a function of hazard and exposure.

Risk = hazard x exposure

Hazard – intrinsic (it is there or it is not) *potential* of a material to cause harm to human health and/or the environment; also synonymous to threat

Exposure – the extent and the duration of or the frequency with which the operator is exposed to the hazard

Biological agent - a micro-organism, cell culture, or human endoparasite, whether or not genetically modified, and products derived from them which may cause infection, allergy, toxicity or otherwise create a hazard to human health and the environment; also synonymous with biohazard

Microorganisms - a microbiological entity, cellular or non-cellular, which is capable of replication or of transferring genetic material

Naturally Occurring vs. Biotechnological Risks

Biological risk can be classified into two broad categories: naturally occurring or human-caused.

Naturally occurring biological risks – includes (1) the emergence of antibiotic resistant bacterial infections (tuberculosis, pneumonia, flu epidemic); (2) naturally emerging pathogens attributed to deforestation (monkey pox, Ebola, Lassa fever); (3) spreading of a zoonosis i.e. infected animal population conveying the disease to humans via direct contact, vector or water/foodstuffs; (4) toxins arising from certain molds and fungi (deoxynivalenol, alflatoxins, ochratoxin a); (5) parasitic infection outbreaks in humans; (6) invasive alien species (plants, animals and microorganism)

Human caused or related biological risks – can be further classified into: (1) deliberately induced risks such as the use of harmful biological agents through warfare or terrorism; and (2) biotechnological risks such as products of traditional cross breeding and selection, mutation and modern biotechnology.

It is noteworthy that many of the biological risks that threaten society are natural in origin. In plants for example, introduction of invasive species has caused direct economic and environmental damage (e.g. in the US, cost of damage of 50,000 non-indigenous species is $137 billion per year, Pimentel et al., 2000) or presents risks to public health (e.g West Nile virus). Arguably, the risks posed by GM crops compared to invasive species are minimal. However, it is widely acknowledged that risks present with biotech crops are no different than those present from traditional breeding (NRC, 1989, Tiedje 1989; OECD, 1992; NRC, 1993; NRC, 2002).

Biological Agents and Risk Groups

Another way to classify biological risks is based on the risk posed by biological agents to the health of the laboratory workers and to human health and the environment upon accidental or intentional release. Biological agents are typically used in research or biomedical laboratories. These include the full range of micro-organisms: bacteria, viruses, fungi, protozoa and multi-cellular parasites. Laboratory associated infections (LAI) has been documented since the beginning of the 20th century. The historical accounts of LAIs, though relatively infrequent and the advent of modern biotechnology raised awareness about the hazards of infectious microorganisms and the risks these posed to laboratory workers who handle them and the community if they escaped from the laboratory.

There are three ways that will bring workers into contact with materials that may pose a biological risk. These are:

1. *exposure as a result of working with biological agents* – areas of work include a microbiology laboratory; greenhouse, animal house; activities include isolation, identification and culture of microorganisms or cells including materials used for genetic

modification, intentional contact with animals and plants and materials that originate from animals and plants as part of planned experimental work.

2. *exposure which does not result from the work itself but is incidental to it, mainly because biological agents are present as contaminants* - areas and activities include farming, refuse collection, sewage treatment, handling human body fluids and excreta; handling materials that may be contaminated by these materials such as hypodermic needles or sewage treatment plant.
3. *exposure which is not a result of the work that you do* – unintentional contact with animals and animal and plant materials or people in the workplace.

The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on the risk criteria/factors described below.

- *Pathogenicity of the agent or its product* - inherent risks of a pathogen are based on factors such as the severity of the disease it causes, its virulence and infectivity; 'disease' caused by agent's products include toxicity, allergenicity, physiological activity (e.g. anti-nutritional).
- *Mode of transmission and host range of the agent* – these are influenced by existing levels of immunity, density and movement of the host population, presence of appropriate vectors and standards of environmental hygiene.
- *Availability of effective preventive measures* - measures may include: prophylaxis by vaccination or antisera; sanitary measures, e.g., food and water hygiene; the control of animal reservoirs or arthropod vectors; the movement of people or animals; and the importation of infected animals or animal products.
- *Availability of effective treatment* - includes passive immunization and post-exposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of emergence of resistant strains.

Other considerations that maybe taken to account in classifying biological agents include

- *Origin/source* – indigenous (native, local) or exotic (foreign, alien); e.g. exotic agents posed higher risks to human health because it may cause more severe infection with no available treatment
- *Ability of the organism to survive* – dormancy or resting period; duration
- *Number/concentration of microorganism* – the higher the number the greater the possibility of infection
- *Nature and route of transmission* – inhalation (dust, aerosol), ingestion (food, drink, saliva), contact (cuts, bites, injection)

The *National Institute of Health, USA (NIH) Guidelines*[5] established a comparable classification of genetically modified hazard agents into a particular risk group using the same criteria indicated above. Many countries, including Bangladesh (*see Annex 1 of the Bangladesh Biosafety Guidelines 2006*), have adopted the WHO and NIH risk group classifications and criteria. The descriptions of the WHO and NIH risk groups are presented in Table 1 below.

Table 1. Classification of Infectious Microorganisms By Risk Group ***(Source: BMBL[6] 5th Edition 2007)***

Risk Group Classification	NIH Guidelines For Research Involving Recombinant DNA Molecules 2002	World Health Organization Laboratory Biosafety Manual 3rd Edition 2004
Risk Group I	Agents that are not associated with disease in healthy adult humans	(No or low individual and community risk). A microorganism that is unlikely to cause human disease or animal disease
Risk Group II	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited.
Risk Group III	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group IV	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

The four risk group classification of biological agents is widely recognized but disagreements exist in allocating agents to a particular risk group. WHO recommends to each country to draw up its own classification by risk group of the agents encountered in that country based on the criteria and other considerations enumerated in points 14 and 15.

Containment and Biosafety Levels

Containment refers to the ability to reduce or eliminate exposure of workers, other persons, and the outside environment to potentially hazardous agents. It is also used to

[5] NIH Guidelines For Research Involving Recombinant DNA Molecules (NIH Guidelines)

[6] Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention

describe safe methods, facilities and equipment for managing infectious materials in the environment where they are being handled or maintained. For experimental plants and animals and microorganisms that are restricted within a chosen outdoor environmental zone of control, the term confinement has been used.

The fundamental elements of containment include the following:

- *Safe laboratory practices and techniques*- the most important element of containment; It refers to strict adherence to standard microbiological practices and techniques which require properly trained personnel, a biosafety or operations manual that identifies the hazards and specifies practices and procedures designed to minimize or eliminate exposures to the hazards.
- *Safety equipment (primary physical barrier or personal protective equipment)* – primary barrier includes biological safety cabinets (BSCs), enclosed containers (e.g. centrifuge cups), and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles.
- *Facility design and construction (secondary physical barrier)* - provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. Special design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory.
- *Biological barriers* - *natural barriers* that limit either: (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment; also include *design of facilities* and *special practices* for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location; used for experiments involving recombinant DNA technology to replace hazardous agents and decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the experimental area.

Various combinations of physical and/or biological barriers along with a constant use of standard practices were used to establish different levels of containment for organisms within laboratories (BSL1 through BSL4) and large scale uses (BL1-LS through BL4-LS), plants (BL1-P through BL4-P) and animals (BL1-N though BL4-N). In all cases, four biosafety levels were established, which provide increasing level of protection to personnel,

environment and the community. Categories of containment and biosafety levels are considered separately and detailed descriptions are found in the NIH Guidelines 2002, BMBL 2007 and Bangladesh Biosafety Guidelines 2006.

It is important to note that the risk groups correlate with but do not equate to biosafety levels. The risk group of an agent should be one factor, to be considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted. A summary of the characteristics of the four BSLs within laboratory is shown in Table 2 below.

Table 2. Summary of Recommended Biosafety Levels For Infectious Agents *(Source: BMBL 5th Edition 2007)*

BSL	Agents	Practices	Primary barriers and safety equipment	Facilities (Secondary barriers)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
2	• Agents associated with human disease • Routes of transmission include precutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infections materials. PPEs*: • Laboratory coasts; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents • PPEs: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text

*PPE = Personal Protective Equipment

Good Laboratory Practice (GLP)

Effective containment and many testing procedures are based on sound laboratory management practices. Many guidance documents refer to these practices in general terms as good laboratory practice ("lower case glp") and more specifically as GLP ("upper case GLP"). The former refers to a set of standards used to accredit testing and calibration laboratories (e.g. ISO/IEC 17025). The latter refers to the OECD Principles of Good Laboratory Practice[7], which sets the standards for specific tests studies. Some countries issue their own versions of the GLP Principles based on the OECD Principles of GLP, incorporated as part of national legislations.

[7] Organization for Economic Co-operation and Development; published by the OECD's Environment Directorate, and most recently revised in 1998

The OECD Principles of GLP describe a "quality system concerned with the *organizational process* and the conditions under which *non-clinical studies* are planned, performed, recorded, archived and reported" (OECD definition). It is concerned with assurance of data quality (sufficient, rigorous, reproducible) rather than the technical validity of the studies undertaken.

Data generated under GLP are designed for product registration, mutual acceptance of data among OECD member countries, and to contribute to protection of human health and the environment. Non-clinical studies include physico-chemical testing, toxicity, mutagenicity, environmental toxicity, bioaccumulation and residue studies; studies of effect on mesocosms[8] and ecosystems, and the analytical chemistry associated with such studies. Test items include synthetic chemicals, items biological in origin and living organisms. A study covers work done in a laboratory, in animal houses, in greenhouses, and in the field.

The GLP Principles prescribes a set of guidelines for the following: test facility organization and personnel, quality assurance program, facilities, apparatus, material, and reagents, test systems, test and reference items, Standard Operating Procedures (SOPs), performance of the study, reporting of study results, storage and retention of records and materials. The elements unique to GLP are as follows: Study Director (and any Principal Investigators), Quality Assurance unit, Standard Operating Procedures (SOPs), Study plans (protocols) and reports and Data archive.

GLP compliance monitoring is required for mutual acceptance of data. Periodic inspection of test facilities and/or auditing of studies are conducted for the purpose of verifying adherence to GLP principles. Compliance and monitoring are conducted by international, regional or national accreditation bodies e.g International Laboratory Accreditation Cooperation (ILAC), Asia Pacific Laboratory Accreditation Cooperation (APLAC), Australia's National Association of Testing Authorities (NATA). Different countries may require different proofs of compliance with regard to GLP requirements.

Risk Analysis[9]: Concepts and Issues

In this document, risk analysis is used in its broadest sense as an integrated process consisting of three major components: **risk assessment, risk management and risk communication.** The individual components are distinct, but are linked to achieve a well-functioning risk analysis process that forms the basis for decision making on any operation or

[8] An experimental system that simulates real-life conditions as closely as possible. For example, a sediment sample contained in an opened vessel and placed on a riverbed or in a flow-through system in a laboratory (not temperature controlled as it should simulate outdoor temperatures). Environment Canada St Lawrence Glossary URL: http://www.qc.ec.gc.ca/csl/glo/glo006_e.html

[9] Information on this section were abstracted from the following references: Bangladesh NBF 2007 and Biosafety Guidelines 2007; Australia Risk Analysis Framework, 2005; FAO/WHO, 1997 and Codex Alimentarius Commission, 2003; Cartagena Protocol on Biosafety 2000; PRRI Guide For Notifications And Risk Assessments For Releases Into The Environment Of Genetically Modified Organisms Module 1: Genetically Modified Crop Plants URL: http://www.pubresreg.org

dealing of GMOs. The use of risk analysis in this manner generally conforms to Bangladesh's proposed NBF and Biosafaty Guidelines, and other national and international principles and guidelines to protect human health and the environment from risks posed by or as a result of modern biotechnology.

The terminologies associated with risk analysis in this section are:

Risk - likelihood that under particular conditions of exposure an intrinsic hazard will represent a threat or harm; risk is a function of hazard and exposure.

Risk = hazard x exposure

Hazard – intrinsic (it is there or it is not) *potential* of a material to cause harm to human health and/or the environment; also synonymous to threat

Exposure – the extent and the duration of or the frequency with which the operator is exposed to the hazard

Likelihood – probability of something happening

Consequence – adverse effects or outcome caused by an event

Stakeholders – individual, groups and institutions who may affect, be affected by, or perceive themselves to be affected by the decision, activity or risk; synonymous with 'interested parties'

Components of Risk Analysis

Risk assessment is the first and the *scientific component* of risk analysis. It is a rigorous science-driven process used to identify a hazard and obtain qualitative or quantitative estimate of the levels of risk posed by a hazard including possible adverse effects to human health and the environment. It typically consists of four steps: (1) hazard analysis (identification and characterization), (2) likelihood estimation, (3) consequence evaluation; and (5) risk estimation. A more detailed discussion of risk assessment is presented in Section and some examples for GM crops are presented in Section 5.

Risk management is the second and *decision-making component* of the process of risk analysis. It is primarily supported by risk assessment but is informed by other risk considerations. Risk management is concerned with evaluating whether the risks identified by the risk assessment process are acceptable and manageable, then selecting and implementing the control measures as appropriate to ensure that risks are minimized or controlled. A more detailed discussion on the methodology of risk management and other considerations is presented in Section 6. A more thorough treatment of the risk management for various uses of GMO and the post-monitoring aspects are presented in a separate Lecture Module.

Risk communications is recognized as the third component that underpins the risk assessment and risk management processes. It is the process of exchange of information and opinions concerning risk and risk-related factors among various stakeholders concerned with risk (Codex definition). It strengthens the over-all process of risk analysis by helping to

define the issues and providing the link and the feedback mechanism that informs the two processes. The principles, structure and process of risk communication presented in Section 7.

Risk analysis applied in the broad sense separates the risk assessment from risk management. The reasons are: to maintain the scientific integrity of the risk assessment, to avoid confusion over the functions to be performed by risk assessors and risk managers, and to minimize any conflict of interest. In practice, however, this separation is rarely clear-cut and variation in its implementation exists among countries and across regulatory institutions.

Principles of risk analysis: general aspects

While regulatory frameworks for risk analysis vary among countries, the underlying general principles in assessing safety from risks posed by GMOs to human health and the environment share many similarities. These include:

Science-based – Risk should be assessed using information obtained through application of science and the scientific method i.e. rigorous and systematic, reproducible, with testable null hypothesis, qualitative and/or quantitative. Methods used should be appropriate and data generated of high quality to withstand scientific scrutiny and peer review.

Open, transparent and documented – All aspects of the process of risk analysis should be documented fully in a transparent manner. Documentation should be accessible to all interested parties, while respecting legitimate concerns to preserve confidentiality. This principle also refers to the selection of experts who will conduct the risk assessment. Experts responsible for risk assessment should be selected on the basis of their expertise, experience, and their independence with regard to the interests involved.

Case-by-case -Risk should be assessed on a case-by-case basis. This means that for each case, the risk assessment methodology and required information may vary in nature and level of detail, depending GMO concerned, its intended use (e.g. laboratory, field , market) and the likely potential receiving environment (e.g. presence of wild relatives, non-target species, endangered species.

Comparative - Risks should be compared to background risks i.e. risks is considered in the context of the risks posed by the non-modified recipients or parental organisms within the context of the intended use. This requires appropriate comparator and well-established baseline information.

Systematic - The risk analysis should follow a structured, step-by-step approach. The key steps are: establish the purpose, scope and boundaries of the risk assessment, assess the risk, and manage the risks.

Iterative - Risks should be evaluated and reviewed as appropriate in the light of newly generated scientific data. Conclusions and assumptions should be examined relative to new information.

Inclusive – The process of risk analysis should be all-encompassing. The three components of risk analysis should be applied within an overarching framework for management of food related risks to human health and the environment. It should draw information from a wide range of credible sources and could also take into account expert advice of, and guidelines developed by, relevant international organizations. Effective communication and consultation with all interested parties should be ensured in all aspect and stages of the process of risk analysis.

The Methodology of Risk Assessment and Risk Management: Key Steps

General guidance on the methodology of risk assessment and risk management exists and they share many similarities. Annex III 8 of the Cartagena Protocol on Biosafety is a good guide and the steps typically followed are enumerated below.

1. *Hazard analysis* - An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;
2. *Likelihood estimation* - An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;
3. *Consequence evaluation* - An evaluation of the consequences should these adverse effects be realized;
4. *Risk estimation* - An estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;
5. *Risk management* – A recommendation as to whether or not the overall risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks, including monitoring.

It should be noted that the level of details and sequence of some of the steps indicated above also vary across countries. More detailed discussion of the methodology of risk assessment and risk management are presented in succeeding sections. (see Section 4-6)

Concepts and Issues in Risk Analysis

There are a number of concepts and issues that are very important in gaining a better understanding of the process of risk analysis. These include:

Concept of Familiarity

Risk assessment of GMOs requires information on the identity, characteristics and history of safe use of the organism that is subjected to genetic modification. Most genetically

modified organisms to date have been developed from organisms that are "familiar" i.e. there is sufficient available information about the organism's attributes, and long history and experience of its safe use.

The concept of familiarity provides a way to recognize the potential risks by using already available information on the attributes of the organism. Because of familiarity, effective methods can de devised to avoid or manage the risks to acceptable levels. For example, it will be possible to determine the potential for invasiveness of the crop based on knowledge of the biology (e.g. presence of traits that are associated with invasiveness) and the presence of wild compatible relatives. Likewise, it will be possible to identify the potential allergenicity of the GMO if knowledge and history of safe use of the origin/source of the gene used in genetic modification is available. In this context, the concept of familiarity is not a risk assessment by itself but a useful tool for identifying, evaluating and managing risks.

Concept of Substantial Equivalence

In assessing the risks posed by GMOs to human health and the environment, the concept of familiarity is used together with the concept of substantial equivalence. Substantial equivalence is based on the principle that GMOs can be compared with their conventional counterparts[10] that have an established history of safe use. The concept is used to identify the similarities and differences (includes intended changes and unintended changes)[11] between the GMO and its conventional counterpart to be able to determine if the GMO is 'as-safe-as' or presents any new or greater risks than its conventional counterpart. The concept of substantial equivalence does not establish absolute level of safety, but relative level of safety.

Internationally, the concept of substantial equivalence is recognized as one of the principles for environmental risk assessment by the Cartagena Protocol on Biosafety, and in food safety assessment by the Codex Alimentarius Commission. The relevant texts (italics provided) are as follows:

Cartagena Protocol on Biosafety (2000)

Annex III 5 –Risk Assessment

Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern

[10] 'Conventional counterpart' means a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food; (Codex Alimentarius Commission, 2003); synonymous with 'traditional counterpart' or' non-transformed counterpart'; 'non-modified recipient or parental organisms'

[11] Intended changes refer to the inserted genes and their related substances and traits; Unintended changes –refer to the pre-differences in pre-determined parameters between the GM plant and its appropriate non-GM comparator(s) e.g. changes in "phenotype" - yield, plant morphology, flowering time, day degrees to maturity, duration of pollen viability, response to plant pathogens and insect pests, sensitivity to abiotic stress

biotechnology, *should be considered in the context of the risks posed by the non-modified recipients or parental organisms* in the likely potential receiving environment.

Codex Alimentarius Commission Principles and Guidelines on Foods Derived from Biotechnology (2003)

Section 3.10 –Principles

Risk assessment includes a safety assessment,…The safety assessment *should include a comparison between the food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences.* If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health.

As an approach, it should be noted that the concept of substantial equivalence is considered a *starting* point for the safety assessment to structure the safety assessment procedure, and focus on the identified differences that may require further testing. Its application is limited by the choice of an appropriate comparator and availability of sufficient scientific information relevant to the risk assessment. These points are illustrated in the three cases presented below.

1. *GMOs that are shown to be substantially equivalent to the conventional counterparts* are regarded as being 'as safe as' their counterpart. No further safety considerations other than those for the counterpart are necessary.
2. *GMOs that are substantially equivalent to the conventional counterpart except for defined differences* need further safety assessment which should focus only on the defined differences. Typically, the defined differences will result from the intended effect of the genetic modification that may, or may not, change the endogenous traits, or produce new traits in the host organism.
3. *GMOs that are not substantially equivalent to the conventional counterpart***.** Up to now and probably for the near future, there have been few examples of these GMOs. Nevertheless, it is conceivable that with future developments in biotechnology, these kinds of GMOs will be produced. In these cases, the concept of substantial equivalency cannot be applied.

As a final note, in addition to the limitations mentioned above, the use of the concept of substantial equivalence in risk assessment has been criticized as subjective, inconsistent and pseudo-scientific (Millstone et al). However, despite its limitations and criticisms, there is wide recognition that the concept of substantial equivalence remains to be the most practical approach currently available to framing the risk assessment process.

The Precautionary Approach

Principle 15 of the Rio Declaration on Environment and Development (UNCED, Rio de Janeiro, June 1992)

"In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

There are a number of important points to keep in mind about Principle 15 of the Rio Declaration in conducting risk analysis.

1. The term 'precautionary approach' is specifically used to differentiate it from the legal connotation of the term 'precautionary principle'. The latter is compulsory or legally binding while the former maybe binding in some cases but normally does not have the same force as a law (Recuerda, 2008). Because it is an 'approach' and not a 'principle', Principle 15 allows for discrimination between countries in applying the approach based on their capability, which a law or principle will not allow. Furthermore, Principle 15 allows other costs (e.g. social or economic) to be considered in order to be cost-effective in applying the approach. In view of these, the 'precautionary approach' is viewed as softening of the 'precautionary principle' (Garcia, 1995).
2. The precautionary principle in the context of Principle 15 explains the idea that scientific uncertainty (i.e. source or form of doubt) should not prohibit using preventive measures to protect the environment; and use of "cost-effective" measures indicates that costs can be considered when applying the approach.
3. Principle 15 identifies the triggers to propose a precautionary approach: 1) a scientifically sound identified threat of damage and 2) there is scientific uncertainty about the extent of the potential adverse effects (PRRI, www.pubresreg.org)
4. Finally, Principle 15 refers to potentially irreversible harm to be the most important application of the precautionary approach. Where risk are irreversible, decision makers will act from the perspectives of prudence and precaution.

Many countries have adopted the same phrasing of Principle 15 of the Rio Declaration in their regulatory systems and have established risk assessment mechanisms based on the precautionary approach. The interpretation and implementation of the precautionary approach vary across countries because they differ in their opinions on thresholds of risk and degree of scientific uncertainty allowed in the process of risk analysis. Many regulatory approaches recognize the imperfect nature of evidence when making decisions. In conformity with the precautionary approach, preventive measures are built in their risk management approaches to allow certain activities with limitations when appropriate.

It is disconcerting to note that over the last years, there seems to be a tendency to interpret the precautionary approach in the excessively conservative way as the precautionary principle i.e. irrespective of possible benefits, a new technology should never be introduced unless there is guarantee that no risk will arise. As Nuffield Council of Ethics (2004) observed, such interpretation is impractical and invokes the fallacy of thinking that the option of doing nothing is itself without risk. In addition to foregone benefit, the members of the

Public Research and Regulation Initiative (PRRI) are concerned that any question about GMO is deemed sufficient to stop research in this field regardless whether any risks (let alone threats of serious or irreversible damage) have been identified (PRRI, www.pubresreg.org).

Uncertainty

Uncertainty is an inherent property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communications (Hayes, 2004). Simply defined, uncertainty is a form or source of doubt. There are five different types of uncertainty that can be applied to risk analysis, which is enumerated below *(Please refer to Appendix D of the Risk Analysis Framework (2005) for more detailed explanation).*

- **epistemic** - uncertainty of knowledge, its acquisition and validation. The most common examples are statistical errors, use of surrogate data (e.g. extrapolation from animal models to humans), incomplete or ambiguous, contested data or unreliable data. Epistemic uncertainty could be reduced by designing more rigorous experiments, applying more powerful statistical analyses and GLP.
- **descriptive** - uncertainty of descriptions that may be in the form of words (linguistic uncertainty), models, figures, pictures or symbols (such as those used in formal logic, geometry and mathematics). Usually associated with qualitative measurements; inconsistent and incomplete definition and application of words. For example the word 'low' may be ambiguously applied to likelihood of harm, magnitude of a harmful outcome and to the overall estimate of risk. Descriptive uncertainty could be reduced by using accurate and consistent definitions and providing clear parameters, scope and boundaries.
- **cognitive** (including bias, perception and sensory uncertainty) Cognitive unreliability can be viewed as guesswork, speculation, wishful thinking, arbitrariness, date, or changeability. One way to reduce cognitive uncertainty is through effective communication strategies.
- **entropic (complexity)** - uncertainty that is associated with the complex nature of dynamic systems such as a cell, an organism, the ecosystem, or physical systems (e.g. the weather). Complexity and incomplete knowledge contribute to inability to establish the complete causal pathway in the system. Consequently, a deterministic system can have unpredictable outcomes because the initial conditions cannot be perfectly specified. Complexity could be reduced by generating more information about the various components and relationships in the system.
- **intrinsic** - uncertainty that expresses the inherent randomness, variability or indeterminacy of a thing, quality or process. Randomness can arise for example from genetic difference. A critical feature of intrinsic **uncertainty** is that it cannot be reduced

by more effort such as more data or more accurate data. In risk management, safety factors and other protective measures are used to cover this type of uncertainty.

The following are examples of uncertainty within the elements of risk analysis (RAF, 2005)

Risk assessment

- uncertainty in the nature of the GMO, such as the lack of knowledge of biochemical properties of the introduced genes, environment- specific performance of the GMO, its interaction with other biological entities and processes, or landscape changes over long time periods;
- uncertainty of the calculations within the risk assessment process, including assessment of hazards, likelihood and consequences;
- uncertainty in descriptions used in qualitative risk assessments due to insufficient explanations of terminology, use of related terms that are not fully congruent or the use of the same term in different contexts.

Risk management

- balancing the sufficiency of protective measures against their effectiveness;
- decision making in the presence of incomplete knowledge and conflicting values.

Risk communication

- uncertainty of communication effectiveness due to difference in knowledge, language, culture, traditions, morals, values and beliefs.

There are a number of ways to address uncertainty in risk analysis of GMOs

1. Request further information on the specific issues of concern. Where there is uncertainty more experiments can be required in order to answer the question. However, it must be recognized that the effort and resources required to acquire greater knowledge increases exponentially with each demand for greater precision or detail. In many instances, these may not be technically (e.g. no valid protocol) or practically (e.g. unaffordable cost) possible.
2. Implement appropriate risk management strategies and/or monitoring the GMO in the receiving environment.
3. In cases where further experimentation may not provide the necessary information, the 'worst case' scenario approach can be applied where the focus is less on determining the likelihood of an occurrence, but rather evaluating what the consequences of the occurrence would be.

Food safety aspects vs environmental aspects

The concepts and issues in risk analysis discussed above apply to risks related to food safety and risk related to the environment. Toxicity and allergenicity are key issues to risks in food safety and risks to human health safety as component of the environment. There are differences between them and it is important to draw these distinctions more clearly to avoid

confusion particularly when addressing the language of the CPB where it mentions "'taking also into account human health". The PRRI Guide (www.pubresreg.org) summarizes these distinctions follows

1. In evaluating the risks of toxicity and allergenicity, here is a difference between looking at toxicity in terms of food safety, where it is assumed that large quantities may be consumed frequently (i.e. scenarios in which even low levels of toxicity may have a consequence) and toxicity in the context of environmental safety, where the focus is on effects of minor consumption (e.g. GMO accidentally eaten during field trial).
2. In evaluating the risks of toxicity and allergenicity as a consequence of exposure, the type of application is taken into account in the case of environmental safety but not in food safety. For example, for small scale field trials, in which the material resulting from the field trial is not consumed by humans or animals, toxicity and allergenicity would generally be of no consequence. For large-scale and market releases, toxicity and allergenicity would be of consequence and therefore needs to be addressed and usually the results of toxicity and allergenicity assessments are included in risk assessment.
4. In looking at toxicity as a result of genetic modification, two aspects need to be distinguished: possible toxicity of the gene product, and, in the specific case of food safety, possible insertion effects that may cause changes in pathways in the plant, including pathways that are related to toxicity. Although the latter case can be compared with the normal effects of genomic rearrangements that happen during plant breeding, it is practice that any such insertion effects, that are applicable to the specific event only, and bear no relation to the transgene, be checked in a food safety assessment, before the crop is placed on the market.

The Process of Risk Analysis: Risk Assessment

Risk assessment is the primary and the *scientific* component of risk analysis. It is a science-driven process of identifying the potential hazards and obtaining qualitative and quantitative estimates of risk by assessing its two components, likelihood and magnitude of any adverse outcome that may arise including the degree of uncertainties in those estimates. Risk assessment can also be described as answering the following questions: *What are the hazards? What are the chances (likelihood) that hazard will occur? What are the potential adverse effects (consequences) if the hazard does occur?* (RAF, 2005)

Risk assessment is a scientific process conducted by experts. It focuses on asking empirical questions about potential risks. It gives more weight to evidence derived from experimental data of testable risks with well formulated hypotheses. It takes care of insufficient information and scientific uncertainty by asking for more evidence, developing better analytical methods and making provisional decisions based on prudence and precaution.

Risk assessment does not deal with speculations. It is not concerned with answering questions on economic, ethical and cultural impacts. These are addressed in the risk management component (see Section 6) of the risk assessment process.

Risk assessment typically consists of four steps: (1) hazard analysis (identification and characterization), (2) likelihood estimation, (3) consequence evaluation; and (5) risk estimation described below.

The Methodology of Risk Assessment

General guidelines and many papers exist on the methodology for assessing risks to people and environment[12]. In this section, Annex III 8 (a-d) of the Cartagena Protocol on Biosafey (2000) is used as guide to enumerate the steps typically followed in risk assessment whether for food or environment. The additional information to help explain each step was abstracted primarily from the Risk Analysis Framework (2005) and PRRI draft guide for risk assessment (www.pubresreg.org).

(1) Hazard analysis (identification and characterization) - An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health (CPB, Annex III 8 (a))

Hazard identification establishes the intrinsic or 'built-in' potential of the biological agent (e.g. GMO or GM foods) to cause harm. Hazard characterization aims to evaluate in qualitative and quantitative terms the nature of the identified intrinsic hazard. Quantitative and qualitative techniques are used in hazard identification (Hayes et al, 2004). Qualitative techniques include checklist, brainstorming, expert consultation, fault and event trees. Quantitative techniques include HAZOP analysis, hierarchical holographic model (HHM), SWOT analysis, Dephi analysis, etc. Approaches to hazard analysis may be inductive (top down) or deductive (bottom up). Checklist and the inductive approach appear to be the status quo of hazard analysis. Evidentiary support could range from unsubstantiated statements (weak evidence) to experimental data (strong evidence).

Hazard analysis also involves establishing the causal link and pathway or route of exposure between hazard and an adverse outcome. It also involves identifying the measurable properties of the hazard in order to accurately assess that harm has occurred (Table 3).

(2) Likelihood estimation- An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism (CPB, Annex III 8 (b))

Likelihood is the probability that the harm will occur. It is expressed as relative measure of frequency (the number of occurrences per unit time) and probability (from zero to one,

[12] As of May 2008, a total of 155 citations on risk assessment and risk management is available through the Biosafety Information Resources Centre of the Biosafety Clearing House

where zero is an impossible outcome and one is a certain outcome). It is important to remember that likelihood estimation is a predictive process. The accuracy of prediction is directly proportional to time of occurrence i.e. short term outcome is more accurately assessed than long term outcome.

Table 3. Examples of potential harm identified and their measurable properties

Hazard	Measurement Attributes
Reduced fitness, increased persistence, invasion	Occurrence and biological properties – traits for weediness and invasiveness;
Toxicity to non-target organisms	Mortality; survival; population morbidity, species richness
Habitat modification- altered bio/geo-chemical cycles	Carbon, nitrogen, phosphorus flux; frequency of floods, fire; pollutant concentration
Loss of biodiversity and extinction of species	Diversity indices; species richness;
Creation of new viruses	Occurrence, number. severity, host range
Human toxicity and allergenicity	Biological, physiological and physical abnormalities; mortality; frequency and age of morticity

Here the term 'estimation' is chosen, because exact numbers of the frequency with which something will happen in nature cannot always be measured. It is possible in certain risk calculations such as non-target risks but more frequently the risk finding is qualitative on the basis of a weight-of-evidence analysis.

Likelihood assessment maybe qualitatively described as follows:

- *Highly likely* - is expected to occur in most circumstances
- *Likely* - could occur in many circumstances
- *Unlikely (Negligible or Effectively zero)* - could occur in some circumstances
- *Highly unlikely* - may occur only in very rare circumstances

For GMOs, the most important factors that contribute to the likelihood that harm will occur are the survival, reproduction, persistence of GMO; and the receiving environment, including its biotic and abiotic attributes.

(3) Consequence evaluation- An evaluation of the consequences should these adverse effects be realized (CPB, Annex III 8 (c))

Consequence evaluation involves characterizing the significance of the adverse outcome if the hazard occurs. The following criteria should be taken into consideration:

- Severity – number, magnitude, scale
- Spatial extent – geographical (local, national, global); organism (individual, population, community, ecosystem)
- Temporal extent – duration and frequency
- Cumulative and Reversibility
- Background risk – risk that may occur in the absence of the stressor (e.g. GMO)

Descriptors of consequence assessment

- *Marginal* - Minimal or no injury except to a few individuals that may require first aid; Minimal or no degradation of the environment
- *Minor* - Slight injury of some people that may require medical treatment; Disruption to biological communities that is reversible and limited in time and space or number of individuals/populations affected
- *Intermediate* - Injury to some people that requires significant medical treatment; Disruption to biological communities that is widespread but reversible or of limited severity
- *Major* - Severe injury to some people that may require hospitalization or may result in death**;** Extensive biological and physical disruption of whole ecosystems, communities or an entire species that persists over time or is not readily reversible.

(4) Risk estimation - An estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized (CPB, Annex III 8 (d))

Risk estimation combines the information on likelihood and consequence of the identified hazard to come up with the risk estimate matrix shown below. As a general rule, risks with moderate and high estimates will invoke the corresponding risk management treatments or control measures.

Descriptors of Risk Estimate

- Negligible - risk is insubstantial and there is no present need to invoke actions for mitigation
- Low - risk is minimal, but may invoke actions for mitigation beyond normal practices
- Moderate - risk is of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective
- High - risk is unacceptable unless actions for mitigation are highly feasible and effective

Finally, in conducting the steps outlined above, the characteristics of the following, depending on the dealing of GMO, could be taken into consideration::

- Recipient, host or parental organisms.
- Inserted genes, sequences and related information about the donor(s) and the transformation system
- The resulting GMO,
- Detection and identification of the GMO
- The intended use (e.g. the scale of the activity - field trial or commercial use)
- The receiving environment.

Information requirement for risk assessment

Risk assessment for the release of GMOs typically takes into consideration the points enumerated above obtained from Annex 9 of the Cartagena Protocol on Biosafety. A more detailed discussion of the various points is presented below. These information were abstracted from the paper of Konig *et al.* (2004) and PRRI Guide of Risk Assessment (www.pubresreg.org)

1. Information on the recipient or parent organism

The type of information on the parent crop that should be gathered at the outset include

a. *identity , phenotypic and agronomic performance* – taxonomic identity (including complete name, family name, genus, species, subspecies, cultivar/ breed/race/isolate, common name, sexually compatible wild relatives); chemical proximate composition and key nutrients and anti-nutrients

b. *geographical distribution/source or origin* – area of cultivation, center of origin and centers of diversity

c. *history of safe use* – any known nutritional, anti nutritional, toxicological, allergenic characteristics or intolerance; importance in the diet including information on preparation, processing, cooking

d. *compositional analysis* – key nutrients, toxins, allergens, anti nutrients, biologically active substances associated with parent and sexually compatible relatives; information both from the literature and from analytical data

The recipient or parent organism refers to the organism into which the genes are introduced through genetic modification methods. The characteristics of the recipient organism guide the choice of test parameters for comparison of the GMO with its non-modified counterpart i.e. it serves as the reference point. Knowledge of the natural variation of the traits in the recipient is essential in interpreting data when comparing the GMO to its non-modified counterpart under different receiving environment. The history of safe use of the parent can provide additional information to help plan the risk assessment strategy e.g. identifying what should be the focus of further assessment

The OECD has been compiling consensus documents on the (1) biological attributes and (2) compositional characteristics for certain crops species. These documents provide excellent sources of relevant information of the parent or recipient crop. Information from these OECD consensus documents have been accepted by biosafety regulatory authorities in some countries.

2. Information on the inserted genes and sequences and related information about the donor(s) and the transformation system.

The information required includes:

a. *Description of donor (s)* – includes classification and taxonomy, evidence of potential toxicity, allergenicity or pathogenicity, history of use and exposure to the donor; where possible, function of any recombinant DNA sequences used in the transformation study

b. *Description of vector DNA* – includes information on source of all genetic elements used to construct and amplify the vector, functions of all genetic elements including coding sequences, promoters and termination signals, vector map with relevant restriction site; proof of absence of vector fragments not intended to be transferred, nucleotide sequence information

c. *Transgene delivery process* – For *Agrobacterium*-mediated transformation the information requirement includes donor strain and any plasmid contained in that strain; for direct transformation method, such as particle gun include absence of contaminating sequences of bacterial chromosomal DNA or other plasmid DNA, vector sequences.

d. *Characterization of introduced DNA* – includes information on number of insertion sites, copy number of the introduced DNA, ends of inserts adjacent to plant genomic DNA; genomic library of each transformed plant line (under discussion), absence of vector backbone; stability of transgene insertion verified over five or more generations

e. *Characterization of insertion site* – information on the junction of the inserted recombinant DNA and the plant genome,

With regard to transformation method, it has been argued that in using *Agrobacterium*, the risk of transfer of random DNA to the plants is relatively small (Gelvin, 2000); Hellens and Mullineux, 2000). The vector with the recombinant DNA is separate from the vector with transfer function and has a recognition site for the transfer-mediating gene products.

With regard to characteristics of the introduced DNA, all inserted *functional genes* are, in principle, relevant to the risk assessment, regardless of whether they are the 'genes of interest' or genes that have 'traveled along' in the process, such as selectable markers. The underlying reason is the possibility of unintended effects due to the presence of the DNA sequence. For example, a gene with a prokaryotic origin of replication (*ori*) will not be expressed in a plant cell but will also be considered in the risk assessment because it may facilitate replication of genes in the – unlikely – event that they are taken up and recovered in a replicable form by a bacterium. *Oris* are abundant in the bacteria found in the digestive tract of humans and animals, for example, and in bacteria present on plants and animals.

Finally, the level of detail required should depend on the nature of the dealing. For example, in the early stages of research and development of the GM product, full molecular characterization are not yet conducted so it can be assumed that the entire construct may have been integrated into the recipient plant. Hence, the risk assessment is conducted on that basis

and risk is managed by strict containment measures (see Section 2). When the activity has moved to confined field trials, more detailed characterization is requested, leading to a full characterization as a required for large scale field trial or commercial/market release. This is all part of the 'case-by-case' and 'step by step' approach of risk analysis.

3. Information on the gene products; recombinant protein and/or metabolites

With certain exceptions like anti-sense DNA, all inserted functional genes transferred to the recipient organism are translated into primary (protein) and secondary (metabolites) gene products. Hence, both are relevant to the risk assessment process. The information required for the gene products are:

a. *Structure, identity and characterization* – includes molecular weight, amino acid sequence, post-translational modification (e.g. level of glycosylation and phosphorylation), immuno-equivalence, activity and specificity of reaction (if gene product is an enzyme), expression levels (recombinant proteins in various plant tissues; changes in levels of inherent crop micro- or macro- nutrients (e.g. Vit A in Golden rice), significant unexpected changes in the levels of substances detected during compositional analysis)

b. *Mode of action/specificity* - mechanism of action (e.g. Bt class of proteins which are toxic to insect but not humans), overview of all relevant metabolic pathways that could be affected by the enzymes' presence or altered levels or substance specificity (e.g. CP4 EPSPS enzyme that confers tolerance to herbicide glyphosate but not affect the biosynthesis of the specific aromatic amino acids of all plants and microorganisms)

c. *Toxicity* – information on documented exposure and history of safe use; results of previous toxicity testing programs; for novel protein/metabolite, information on structure and function and toxicity tests are required;

d. *Allergenicity* – changes in the characteristics or levels of expression of endogenous allergenic proteins

Toxicity and allergenicity of the gene products are the primary concerns and focus of risk assessment particulary for GMOs that will be used as food/feed. From the perspective of food/feed safety, it is widely recognized that proteins are not generally toxic when consumed orally as it is largely part of human and animal diet. However, almost all allergens are protein. With regard to toxicity, safety concerns and the amount of new data that will be required should be carefully considered in the light of existing information on the protein/metabolite prevalence, similarity to proteins/metabolites that are routinely used by humans and animals, and history of exposure. Safety concerns and new data requirement should be lower in the case of proteins that have no history of adverse effects to humans and animals. With regard to allergenicity, the amount of new data required should take into account the following key considerations: (a) Is the recombinant protein derived from an allergenic source or known

allergen; able to induce de novo sensitization; cross-reactive with IgE antibodies raised by known allergens?; (b) Has transformation altered the allergenic properties of the product derived from the GMO?.

4. Information on the resulting GMO

Information requirement for the resulting GMO includes: (1) Identity, phenotypic and agronomic analysis; (2) Compositional analysis and (3) Safety analysis (animal studies). The information from these analyses is obtained in comparison with the non-Gm counterpart. These analyses focus on detecting any indicative differences in test parameters such as agronomic performance, compositional and nutritional values, and dietary sub-chronic response in animal feeding studies.

Sources of data to enable detailed comparison can come from a variety of sources. Data about the resulting GMO are available from growing the GMO in growth chambers, greenhouses and/or earlier field trials. Field trials are usually undertaken under a diversity of environmental conditions representative of those typical for planned commercial growing. Another major source of data are databases on existing food composition, chemical analyses, toxicology tests. Data can also be obtained from the Biosafety Clearing House for information on field and commercial releases of the same GMOs in various locations.

Detection and identification methods are important in hazard identification, characterization. In various stages of research, development and release of a GMO, molecular characterization and toxicological tests are conducted to generate information on the characteristics of the inserted DNA sequences, the gene products, and the resulting GMO. This means that detection, identification and test methods focusing on the inserted DNA, the resulting proteins and the resulting GMO are crucial.

Examples of currently available DNA based studies widely used include

- Southern blot
- Qualitative PCR
- Quantitative end-point PCR

Protein based testing methods include:

- Western blot
- ELISA
- Lateral flow strip
- Magnetic particles
- Protein chips

Toxicology test methods include

- in vivo and in vitro test systems
- chronic toxicity, carcinogenicity and reproduction studies
- acute animal toxicity studies

Each of these methods has its own advantages and disadvantages in terms of targets, ease of use, specificity, sensitivity, costs, etc. Existing methods have proved to be adequate for the safety of the GMOs that are currently available in the market. Development in the area of detection and testing are being pursued to improve existing techniques and address safety of next generation products of modern biotechnology.

Note: For more detailed discussion on DNA detection techniques, please refer to Lecture Module: Agricultural Biotechnology.

5. Information relating to the intended use

In this document, intended use refers to the definition of 'Deal with GMOs' adopted from the Bangladesh National Biosafety Framework (Chapter 3.5.3). Deal or intended use encompasses a wide range of activities and application. These include: (a) conduct experiments with GMOs; (b) make, develop, produce or manufacture GMOs; (c) breed GMOs; (d) propagate GMOs; (e) use GMOs in the course of development or manufacture of a thing that is not GMOs; and (f) grow, raise or culture GMOs.

These activities and application can be classified also into two categories: (1) 'contained use'; and (2) 'release to the environment'. "Contained use" means any operation undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment (*CPB definition*).

'Release into the environment', in this document, refers to non-contained use activities with GMOs. In many regulatory systems, this means any trial conducted in the field irrespective of scale and availability of confinement measures and commercial release (e.g. seed production). The major distinction between commercial release and field trials is that with field trials, the GMO involved is still under various degrees of control, whereas after placing GMO on the market for commercial production, its use is in principle unrestricted except for specific product-use conditions, such as labeling or monitoring.

6. Receiving environment

The characteristics of the receiving environment are crucial for the risk assessment. For field trials, the information requirement includes the specific physical location of the trial taking into consideration the following relevant characteristics:

- comparison between the normal growing environment with proposed environment for release
- specific environmental factors influencing survival and distribution (e.g. climate, soil conditions)
- presence of sexually compatible crops
- presence of sexually compatible wild relatives

Environmental Risk Assessment of GM Crops: Some Examples

Potential Environmental Risks of GM Crops

GM crops and foods derived from them are the most widely used GMO in commercial use. Both have provoked fierce debates because of concerns on the potential risk they pose to human health and the environment. Many papers and reviews[13] have been written on the potential risks of GM crops and GM foods. In general, the public are primarily concerned with the possible health effects of consuming GM foods, but are also concerned about non-risk issues such as ethics of genetic modification and labeling of foods with GM ingredients.

Issues of concern to the environment include: gene flow risks i.e. reduction in ecological fitness, invasion; persistence; loss of genetic diversity; loss of biodiversity; creation of new viruses; toxicity to non-target organisms; increased use of chemicals in agriculture; food web-modification; altered farming practices; habitat modification etc. The potential risk issues posed by GM crops vary depending on the local conditions.

Some of the currently debated potential environmental risks of GM crops are discussed in the PRRI guide (www.pubresreg.org) and outlined below.

(1) ***Invasiveness /Weediness*****:** Can the inserted gene/sequence cause changes in the weedy characteristics of the recipient plant, i.e. can the recipient – due to the genetic modification - become more persistent in agricultural habitats or more invasive in natural habitat? This could be the case when the inserted gene or sequences confer a selective advantage or changes in fitness or dispersal. Weediness of a plant depends on many different characteristics, such as persistence, outcrossing, dispersal, etc. and other factors such as the receiving environment and its climate.

(2) ***Effects on non-target organisms***. Can the inserted gene/sequence cause adverse effects on populations of non-target organisms, for example by indirect effects on population level of other insects than the target insect or, where applicable, predators, competitors, herbivores, pollinators, symbionts, parasites and pathogens?

(3) ***Unintended effects on the target organisms***: Can the inserted gene/sequence cause unintended adverse effects on the target organisms, such as resistance development? Resistance development is not an adverse effect in itself, unless it impairs other types of treatments such as spraying with microbial pesticides.

(4) ***Toxicity****:* This focuses on the question of whether the expressed product of inserted gene/sequence can result in toxic effects in the recipient plant, and thus become a risk in case of incidental (or insignificant) consumption by humans or animals e.g. example in

[13] P. Lemmaux (2008) reviews some general and food issues raised regarding GE crops and foods and cite peer-reviewed scientific literature, where possible in response to the issues. Annu. Rev. Plant Biol. 2008. 59:771–812. Issues related to environmental and socioeconomic aspects of GE crops and foods will be covered in another review to be published in 2009.

the case when someone has taken by accident a maize cob from a test field. The exposure in the case of incidental consumption will be very low.

(5) ***Allergenicity****:* Similarly to the consideration of toxicity, this focuses on the question of whether the inserted gene/sequence can result in allergenic effects arising from cases of incidental consumption of the GMO by humans or animals, or in case of exposure to parts of the plants, such as pollen.

(6) ***Altered farming practice***. Can the inserted gene/sequence result in a change in management of the genetically modified crop plant that has a negative impact on the environment. For example, can the inserted gene/sequence cause adverse *changes in biogeochemical processes*, such as changes in the nitrogen cycle?

(7) ***Other unintended adverse effects.*** For example, can the inserted gene/sequence reduce effectiveness of an antibiotic used in medicine as result of horizontal transfer of antibiotic-resistance genes

(8) ***Creation of new viruses***. Can the inserted gene/sequence result in development of new virus strains due to the introduction of viral sequences in a plant genome and possible recombination of genetic material?

Environmental risk assessment and food/feed safety assessment

A comparison between environmental risk assessment and food/feed safety assessment is summarized below.

Similarities: The methodology and information requirements for food safety and environmental risk assessments discussed above are basically similar. In terms of information requirement, both assessments give importance to the relevant characteristics of host and insert, the intended changes from new gene product and/or resulting trait and possible unintended changes. With regard to process, both assessments are done in a step-by-step fashion; early steps in the assessment indicate whether or not additional information or testing is required and uses the substantial equivalence approach to compare any changes and effects of the GM with the appropriate non-GM comparator(s).

Differences: The safety assessment of GM foods is conducted only in the final product. Food safety assessment is descriptive (i.e. what changes happened). Safety assessment generally investigates toxicity, allegenicity, compositional changes in substance with nutritional and toxic properties, stability of the inserted gene, nutritional effects associated with genetic modification and unintended effects which could result from the gene insertion.

Environmental risk assessment (ERA) covers both the GMO and the receiving environment. The assessment process focuses on evaluation of the genotypic and phenotypic characteristics of the GMO and its effect and stability in the environment. The ecological characteristics of the environment where the GMO will be introduced are also evaluated.

Environmental risk assessments are conducted in all stages of development of the GMO. These ERAs involve a stage approach consisting of

- Laboratory/Greenhouse –with stringent physical containment conditions
- Confined field trial - with conditions that ensure the release is limited and controlled in space and time
- Large scale field trial /Commercial release - with or without specific controls
- Post-commercial release – post-release monitoring requirement

At every stage of this process, scientific knowledge is generated and experiences are gained. This staged approach combined with the appropriate protective measure enable the process to build up the body of evidence if the GMO poses any risks while at same time minimizes exposure to harm of people and the environment.

Environmental risk assessment: some examples

Example 1. PRRI draft guide on environmental risk assessment (www.pubresreg.com)

As indicated in the Section 4, the PRRI Guide on environmental risk assessment methodology typically follows the following steps:

- Hazard identification
- Likelihood estimation
- Consequence evaluation, including a baseline assessment
- Risk estimation

These steps are executed in a phased approach

Phase 1: Consideration of each of the inserted genes and sequences individually

Phase 2: Consideration of the whole plant, including potential synergistic and of possible insertion effects and including available empirical information on the resulting GMO

Phase 1: Consideration of the inserted genes and sequences individually

Step 1. Hazard identification

- Hazard identification step addresses explicitly three closely related topics:
  - the ‘triggers’, i.e. which new genotypic or phenotypic characteristics of the GM plant may cause adverse effects on the environment,
  - the scientifically conceivable scenarios that – in theory - could lead to those adverse effects,
  - a clear description of those adverse effects. For example, it is not helpful to just refer to ‘potential impacts on biodiversity’, because that as such doesn’t clarify the issue at hand.

Step 2. Estimation of likelihood

The next step in the risk assessment is an estimation of the likelihood of a certain inserted gene or sequence actually having a potential adverse effect is influenced by many different factors, such as:

- The characteristics of the inserted gene
- The characteristics of the recipient organism
- The characteristics or the scale of the activity: For example, the likelihood of a genetically modified plant with a certain 'built-in' pesticide resulting in significant impact on insects or other organisms other than the target pest, is negligible in a small-scale confined field trial, but may be likely in wide spread commercial use

In cases where the estimation of likelihood does not result in a clear conclusion, it is sometimes advisable to proceed to the next step of the assessment, by assuming as a 'worst case scenario' that a certain event will occur. The attention is then focused on the next step in the risk assessment, i.e. what are the potential consequences

Step 3. Evaluation of the consequences

This step evaluates the severity of a certain effect in a particular situation and environment. Something that may be of no significant consequence in one environment may be of significant consequence in another.

Evaluating the consequences that the introduction of a genetically modified plant may have on the environment is less straightforward because

1 types of effects that may have to be considered differ strongly from each other, such as weediness, effects on non-target organisms, etc.
2 ecosystems in general are very dynamic systems in which many changes occur constantly.
3 severity of a certain effect has to be compared with the effects of using the non-modified host organism.
4 in the case of introducing a GM variety, it should also be considered that every agricultural activity has an impact on the environment in which it takes place.

In order to evaluate the possible consequences of the introduction of a GMO in the context of these dynamic processes, the concept of "base line" plays an important role.

Step 4. Estimation of risk.

The next step in the risk assessment is the evaluation of risk, for each of the identified potential adverse effects. As explained in Section 4, risk follows from the combination of the severity of a potential adverse effect (i.e. consequence) and the likelihood of it occurring. In

the absence of quantitative descriptions of likelihood, terms often used in this step of the risk assessment are: high, moderate, low, negligible.

It is strongly recommended that steps 1 – 4 above be carried out in a systematic way for each inserted gene or sequence.

To facilitate a systematic approach, matrices such as the one shown below can be used. The use of such matrices helps to focus the assessment, and once a matrix is filled in properly, it can be used to formulate the text into the section 'risk assessment' of the notification.

Annex II - worksheet (risk assessment per gene)

Dossier/Applicant:
Plant:
Type of use:
Gene:

Identified potential adverse effect(s)	Estimation of likelihood	Evaluation of consequence	Estimation of the risk
	Terms used: Highly likely Likely Unlikely Highly unlikely	Terms used: Major Intermediate Minor Marginal	Term used: High Moderate Low Negligible
Potential adverse effect 1			
Potential adverse effect 2			
Potential adverse effect 3			
Etc			

Phase 2: Consideration of the GM plant 'as a whole.

After the systematic 'gene by gene approach', the risk assessment moves to a more 'holistic' phase by looking at the plant 'as a whole'. In this phase, the risk assessment looks at:

1. ***Potential synergistic effects of the inserted genes*** – the introduced traits confer characteristics that may enhance or reduce the effect of the GM plant in the environment; certain combinations of traits may enhance the potential for adverse effects, whereas other combinations may reduce the likelihood of adverse effects e.g two different Bt genes, for example, is sometimes applied to reduce the likelihood of resistance development in the target organism

2. ***Available data of the GMO itself, including data on insertion effects*** - data about the resulting GMO are available from growing the GMO in growth chambers, greenhouses and/or earlier field trials. Possible insertion effects as a result of insertion of a sequence within a gene, which could interfere with the pathways in the plant is checked before the crop is placed on the market.

   The example shown below was abstracted from Annex 4 of the PRRI Guide (http://wwwpubresreg.org**)** to illustrate the steps in the risk assessment methodology described above and in Section 4. In this example, two potential adverse effects of the environmental release of GM crop with Bt gene are identified and assessed using the risk assessment methodology outlined in Section 4.

Example of risk assessment considerations for releases of GM crop plant with a Bt gene (e.g. CRY1AB, CRY1AC, CRY1FA, CRY2AB)

Identification of potential adverse effect	Estimation of likelihood	Evaluation of consequence	Estimation of the risk
Potential effects on non target organisms			
Potential unintended effects on the target organism			
Etc.			

The different considerations for two different cases are discussed below:

1. Potential effects on non-target organisms

- ***Hazard identification***
  - Trigger: the inserted genes code for insecticidal toxins; risk assessment should include question of potential effects on non-target organisms
  - Scenarios that would be considered are
    - 1) direct effects in the case of other insects or other animals eating the GM plants with the Bt gene, and
    - 2) indirect effects in the case of other animals consuming the target insects.

In the latter case there may be different types of effects, either because a) those other organisms could ingest indirectly the Bt toxin, or b) because those other organisms would have – if the Bt toxin is effective – fewer insects to prey on. Point of debate under scenario b) is the fact that large numbers of insects caused by crop fields are not a natural situation.

- ***Estimation of likelihood:*** In GM crops with Bt genes to date, the gene products are well known to be highly specific and limited to a small group of Lepidoptera. The likelihood of those Lepidoptera insects being directly affected by the Bt toxin depends first of all on the type of activity,
    - Small scale field trials, any impact on the population level of those Lepidoptera insects is very unlikely
    - Large scale commercial use, the estimation of likelihood considers the presence and feeding behaviour of those Lepidoptera insects, which depends on those insects and on the crops involved. When those insects are not present in the area of planting or do not use the crop involved as main source of food, then an impact on the population level of those insects is very unlikely. When they are present and do use the crop involved as main source of food, then additional testing may be required.
- ***Evaluation of consequence*****:** If the empirical testing results show a significant impact on the population level of those other Lepidoptera insects when exposed to GM crops with Bt Toxin, then an evaluation of the consequence will follow. Impact of growing GM crops will be
    - Intermediate or major –if those other insects are threatened species
    - Minor or marginal –if those insects are widely available in the country.
    - Any impact may even be welcome - if the other insects are also pest insects,

The results of any testing this stage needs to be compared with a proper baseline, derived from growing the unmodified recipient plant.

- ***Estimation of risk.*** If the evaluation of the consequences shows that the consequences are not marginal, then the estimation of risk will follow, and will depend on the outcome of the estimation of the likelihood and the evaluation of the consequence. What that estimation finally will be, will vary from case to case.

If for example a certain crop is normally grown on a very large scale in a country, then the conclusion may be different then when a crop is only marginally grown. This part of the risk assessment can also make use of available data resulting from growing Bt crops on a commercial scale. To date, no verifiable reports have been produced of direct effects on non-target organisms in areas where Bt crops are grown.[14]

The conclusion of the risk assessment may be high, moderate, low or negligible.

[14] Note: The October 2005 issue of Environmental Entomology introduces a new section "Transgenic Plants and Insects" with 13 papers on the longer-term assessment of potential non target effects of transgenic Bt cotton and corn active against lepidopteran and coleopteran pests.
http://titania.esa.catchword.org/vl=3626685/cl=31/nw=1/rpsv/cw/vhosts/esa/0046225x/latest.htm.
The articles are listed in endnote of this Guide (www.pubresreg.org)

- ***Consideration of risk management.*** As discussed under 'likelihood',
    - in cases of small-scale confined releases, the risk management applied by confinement is usually sufficient to address the issue of effects on non-target insects.
    - In case of large scale, commercial use whereby the estimation of the risk of effects on certain non-target organisms is not negligible, the next step is normally to consider risk management strategies. However, it is obvious that in the case of commercial use of a crop risk management aimed at preventing certain insects to forage on the crop is practically not feasible. In those cases, the risk assessment moves ahead to the next stage

2. Potential unintended effects on the target organism-resistance development against Bt

- ***Hazard identification***

- ***Trigger:*** Resistance development against Bt is in itself not an adverse environmental effect but an adverse agronomic and commercial effect. However, it can also be an environmental effect, in case it impairs other treatments such as spraying with microbial pesticides. Whether or not this may be the case depends on the pest insect and crop involved. For example in the case of the European Corn Borer in maize, microbial treatments are not widely used and therefore there would be no trigger for further examination from a biosafety point of view, whereas in potato and rice microbial pesticides are used.

- ***Estimation of likelihood:*** in the case of small scale field trials, the likelihood of resistance development is very, very low. In the case of commercial use, the likelihood can be high in cases whereby a certain Bt crop would be grown for long periods on large areas without effective resistance development strategies.
- ***Evaluation of consequence:*** The severity of the environmental consequence of resistant development will depend on the extent to which treatments such as microbial pesticides are used. The larger the area of microbial pesticide use, the more sever the consequence.
- *Estimation of risk.* The estimation of the risk of resistance development will depend on the likelihood of resistance development (which may be different for each type of Bt) and which depends on the availability of resistance management strategies.

Example 2. James Hancock 2003. A framework for assessing the risk of transgenic crops Bioscience 53:512-519

Underlying assumption:

- Potential invasiveness of a plant species can be predicted if we have knowledge about its biology, its distribution and the likely fitness impact of a transgene

Features of the approach

- Scientifically sound—approach supported by Tiedje et al. (1989); U.S. National Research Council (2000)
- Makes use of already-existing scientific information
- Flora of specific regions
- Existing information about the mode of action of the transgenes
- Identifies data gaps that must be filled by new research submitted by an applicant

Assessment Steps

- **Step 1.** Classify species according to invasive biology. The six species categories are shown in Table 1
- **Step 2.** Assess relative fitness impact of transgenes. The five transgene categories are presented in Table 2.
- **Step 3.** Combine species category with transgene category to determine if the crops are safe for release without further experimentation (Table 3) or need additional experiments (Table 4)

Table 1. Invasive biology of crop species and theor compatible relatives in North America

Category	Biology	Examples of crops in North America
S-1.	No compatible relatives. Crop carries only a few weediness traits and does not escape or persist	Broccoli, cabbage, cauliflower, citrus, cucumber, cotton, eggplant, pea, potato, soybean, sugarcane, tomato, and watermelon
S-2	No compatible relatives. Crop carries an intermediate number of weedy traits but rarely escapes and does not persist.	Peanut and beans
S-3	No compatible wild relatives. Crop carries many weediness traits and can escape and persist.	Barley and wheat
S-4	Has compatible relative. Crop or relative carries only a few weediness traits. Crop can escape but does not persist. Native relative does not aggressively spread.	Celery, lettuce, maize, melon, pepper, squash, and tobacco
S-5	Has compatible relative. Crop or relative carries intermediate numbers of weedy traits. Crop can escape and persist. Native relative does not aggressively spread.	Apple, asparagus, beet, blueberry, carrot, cranberry, onion, pear, poplar, plum, radish, spruce, and strawberry
S-6	Has compatible wild relative. Crop or relative carries many weediness traits. Crop can escape and persist. Native relative spreads aggressively.	Oats, rapeseed, rice, sorghum, and sunflower

Table 2. Relative fitness impact of transgenes

Category	Fitness Impact	Examples of transgene use
T-A.	Neutral in the native environment	Marker genes
T-B	Detrimental in the native environment	Male sterility, altered fiber quality, altered fruit ripening, and storage
T-C	Variable, depending on invasiveness of crop or native	Herbicide resistance
T-D	Variable, depending on level of biological control	Viral, fungal, and pest resistance
T-E	Potentially advantageous in the native environment	Cold, drought, and metal tolerance; improved nutrient uptake; altered development

(a) Crops safe to release without further experimentation

Table 3. Transgenic crops safe to release without further experiment

Crop Category	Transgene Category	Examples of crops in North America
S-1.	T-A, T-B, T-C, T-D, T-E	There are no compatible relatives, and the crop has so few weediness traits that even the most dramatic changes in phenotype are highly unlikely to make it invasive. The crop is easy to control without herbicides.
S-2	T-A, T-B, T-C	There are no compatible relatives, but the crop has enough weediness traits that a dramatic change in its adaptations could make it invasive. A lack of pest resistance is unlikely to be the change in its adaptations could make it invasive. The crop is easy to control without herbicides.
S-3	T-A, T-B	There are no compatible relatives, but the crop itself is weedy, so any change in its fitness might make it more invasive. Herbicides are also needed for the crop's control.
S-4	T-A, T-C	The crop or native relative has so few weediness traits that even the most dramatic changes in phenotype are highly unlikely to make it invasive in agronomic systems; however, advantageous and detrimental transgenes could escape into natural populations and significantly alter their fitness. It is relatively easy to control the crop and its relatives without herbicides.
S-5	T-A, T-B, T-C	The crop or native relative has enough weediness traits that a dramatic change in their adaptations could make it invasive; however, advantageous transgenes could escape into natural populations and positively alter their fitness. The escape of detrimental traits is unlikely to have long-term influences on native populations, because population sizes are large. It is relatively easy to control the crop and its relatives without herbicides.
S-6	T-A, T-B	The crop is already invasive, so any positive change in its fitness might make it a greater pest. Beneficial transgenes associated with environmental tolerances and pest resistance could escape into natural populations and alter their fitness. The escape of detrimental traits is unlikely to have long-term influences on native populations, because population sizes are large. In addition, herbicides are needed for the control of the crop and its relatives.

(b) More data needed

Table 4. Various crop-transgene combinations that require further experimentation

Transgene Category	Crop type	Further research needed
T-A.	None	None
T-B	S-4, S-5, S-6	Document that wild recipient species is not endangered.
T-C	S-3, S-6	Document that any wild recipient species can still be controlled as agronomic weed.
T-D	S-1, S-2	Show that a similar phenotype exists in wild populations. If not, measure levels of native biological control. If levels are significant, test fitness of transgenic crop and hybrids in representative native environments.
T-E	S-1	Show that a similar phenotype exists in wild populations. If not, test fitness of transgenic crop and hybrids in representative native environments.

The Process of Risk Analysis: Risk Management

Risk management is the second and the decision-making component of the process of risk analysis. Risk management is defined as "the process of weighing policy alternatives to mitigate risks in the light of risk assessment, and, if required, selecting and implementing appropriate control options, including regulatory measures (FAO/WHO, 1995; 1997). Its objective is to determine which risks require management and how these risks can be effectively managed or controlled so that its goal of ensuring adequate protection for people and environment is attained.

Risk management is primarily supported by the results of the risk assessment process but may consider risks in a wider context. This allows the risk manager or designated national competent authorit(ies) to take into consideration other inputs e.g. socio-economic considerations (if allowed by regulation) from other interested parties concerned with risks, in the final decision on any dealing of GMOs. This makes risk assessment essentially a political process.

To maintain the scientific integrity of the risk assessment process, it is important to keep the conceptual separation between risk assessment and risk management.

The Key Steps in Risk Management

Risk management is also a step-by-step process which consists of:

(1) ***Risk evaluation***. This step decides whether the identified risk is manageable i.e. a consideration of appropriate risk management strategies

As discussed in Section 4, the rigorous scientific process of risk assessment process ends in a risk estimate. Risk evaluation starts from the result of the risk estimation step. In cases whereby, on the basis of the risk estimation step, the risks involved are not deemed to be 'negligible' or 'marginal', the risk assessment continues with this step, which is a consideration of whether the identified risk is manageable or acceptable. The question to

address is whether the identified risks require specific risk management measures. If the answer is 'yes', then a risk management strategy is defined in the next step. For example, risks with estimates of high or moderate would generally invoke a requirement for management.

Risk evaluation serves as the vital link between risk assessment and risk management. In practice, the functional separation between risk management and risk assessment is less clear in this step.

(2) ***Risk mitigation***. This step is central to the risk management process. It determines the options and plans to reduce or avoid the risks. For cases where a risk management strategy has been defined, the risk assessment 'loops back' to the earlier steps in the risk assessment to determine whether the proposed risk management strategies sufficiently reduce the likelihood or the consequence. This is one reason why risk assessment is often called an "iterative process". Availability of new data, derived for instance from a field confined, 'risk managed', field experiment may also be a reason to revisit and possibly revise a risk assessment

Depending on the case, risk mitigation measures or options may include

- specifying the appropriate containment facilities and biosafety levels (please see Section 2), the conditions for use, handling, storage, transport and disposal; for genetically modified plants: reproductive isolation; by removing of flowers, use of isolation distances or border rows, temporal isolation etc., reduction of the size or duration of an application special design features such as male sterility
- requiring submission of contingency or emergency plans;
- monitoring and surveillance
- GMO detection (for details, please see Lecture Module 1, DNA detection)
- labelling (voluntary or mandatory)

NOTE: *Detailed information on all aspects of monitoring, surveillance, emergency planning are presented in Lecture Module: Use of GMOs Under Containment, Confined and Limited Field Trials and Post Release Monitoring of GMOs*

Countries have put up their own guidelines for dealings on GMOs but there are still no internationally agreed guidelines, except for containment, on exactly how these risk management measure are designed and implemented. Efforts are underway to standardize and harmonize the guidelines on these various risk management measures.

3. Selecting and implementing the most appropriate option(s) and actions.

This step refers to the final decision making process that will ultimately lead to authorization and issuance, or not, of the license required for any dealing of GMO. The risk mitigation measures identified are included as part of the license conditions.

Final decisions are based primarily on the results of the scientific process of risk assessment. However, in this step, the risk management process may take into account other non-risk issues (e.g. socio-economic considerations) and other risk-related factors (e.g. risk perceptions) from various stakeholders to inspire confidence and lead to wider acceptance of the decision. These stakeholders have diverse views and may have conflicting interests. Decision makers need to balance the individual rights of different stakeholders with the need to protect human health and the environment from the adverse effects of unacceptable risks. This step is makes the risk management process essentially a political process.

Risk Management and Socio-economic Considerations

Attitudes towards food and the environment vary widely across societies. In addition to providing the basic need of sustenance, clothing and shelter, food and nature have economic, cultural, ethical and religious meanings and importance to people. Perceived risks to food and environment, including those posed by GMO, is hardly tolerated unless understood and acceptable levels of safety are assured. Scientific assessment of safety undertaken by experts is important, but decision-makers are expected to take into account inputs of various stakeholders concerned with risks, which include socio-economic considerations. Socio-economic considerations cover a wide range of issues and concerns such as advantages vs disadvantages, risk-benefit, costs including cost of doing nothing, respect for cultural diversity, etc.

There are two relevant international documents for considering socio-economic considerations in decision making with regard to potential risks of GMOs to people and environment. These are: (a) Cartagena Protocol on Biosafety of the Convention on Biological Diversity; and (b) Codex Alimentarius (international food code).

Article 26 of the Cartagena Protocol on Biosafety, in particular paragraph 1 states that

"1. The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities."

It is clear in Article 26 of the CPB that countries may to take into account socio-economic considerations in making decisions with regards to GMOs. Paragraph 1 of Article 26 defined the limits and conditions when applying socio-economic considerations in decision-making on risk posed by GMOs to the environment. The definition implies that not all socio-economic considerations can be considered, but only those where GMO directly impacts biodiversity. It also specifies the condition that when countries decide to take into account socioeconomic conditions in decisions on GMOs, it "must be done in a manner that is consistent with other international obligations" which includes the World Trade Organization (WTO).

Codex Alimentarious guidance documents also states that socio-economic considerations may be taken into account in decisions on GMOs. Unlike the CPB, Codex principles are not legally binding to national legislations. However, Codex principles are referred to specifically in the Sanitary and Phytosanitary Agreement (SPS) of the WTO, which is a legally binding international treaty also signed by many countries.

Codex principles on risk management particularly relevant to socio-economic consideration include Section 3.16 of Codex Alimentarius for food derived from modern biotechnology (2003), which states that

> "Risk management measures for foods derived from modern biotechnology should be proportional to the risk, based on the outcome of the risk assessment and, where relevant, taking into account other legitimate factors in accordance with the general decisions of the Codex Alimentarius Commission as well as the Codex Working Principles for Risk Analysis."

Appendix IV of the Codex Working Principles for Risk Analysis on human health (Codex, 2003) and the Criteria for the Consideration of the Other Factors Referred to in the Second Statement of Principles (13) outlines the points and criteria relevant to socio-economic considerations. These include

- other legitimate factors relevant for the health protection of consumers and for the promotion of fair practices in food trade based on the following criteria (Point 28)
  - *other factors should not affect the scientific basis of risk analysis*
  - *other factors which can be accepted on a world-wide basis, or on a regional basis*
  - *specific other factors should be determined on a case-by-case basis*
  - *other factor should consider the feasibility of risk management options concerns related to economic interests and trade issues*
  - *other factors should not create unjustified barriers to trade*

- ***Risk management process should:***
  - take into account an assessment of their potential advantages and disadvantages (Point 34)
  - consider the economic consequences and feasibility of risk management options, giving particular attention to the circumstances of developing countries (Point 35)

As can be noted in the above, the existing guidance documents treat socio-economic considerations in general terms. To date, there are is still no international agreed definition and scope of socio-economic considerations and methodologies for analysis and incorporating socio-economic considerations into the decision-making process. Even at the national level

and for what maybe considered as 'legitimate factor' like economic risk- benefit analysis, there are no biosafety regulatory systems that have formally included a benefits assessment within their regulatory structure. It will take resources and resolve before consensus is reached to effectively incorporate socio-economic considerations in biosafety regulations in many countries.

The Process of Risk Analysis: Risk Communication

Risk communication is the third component of the risk analysis process. Risk communication is defined as "the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among individuals, groups, institutions concerned with risk, including the explanation of risk assessment findings and the basis of risk management decisions." (Codex Alimentarius Commission, 2003). Risk communication in this sense is also addressed in Article 23 of the Cartagena Procotol on Biosafety on public awareness and public participation which states that

1. The Parties shall: (a) Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of living modified organisms in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. In doing so, the Parties shall cooperate, as appropriate, with other States and international bodies; (b) Endeavour to ensure that public awareness and education encompass access to information on living modified organisms identified in accordance with this Protocol that may be imported.
2. The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding living modified organisms and shall make the results of such decisions available to the public, while respecting confidential information in accordance with Article 21.
3. Each Party shall endeavour to inform its public about the means of public access to the Biosafety Clearing-House.

There is wide agreement that effective risk communication is essential at all phases of risk assessment and risk management. It is also recognized that risk communication involves not only risk assessors and risk managers, but also other interested parties like government, industry, academia, consumers, public interest groups and individuals concerned with risk.

Risk communication is also recognized to be an integral part of the risk analysis process. It is embedded throughout the risk assessment and decision making process; two key steps – hazard identification and selection of risk management measures – require effective risk communication to help build trust, reduce conflicts and achieve desired outcomes. In hazard identification, the views and opinions of interested parties about the potential hazards can help define the issues of concern and reduce potential points of conflicts. During the selection of

risk management options, the risk managers may need to consider factors in addition to science in the evaluation of a risk. This should involve active participation of stakeholders and other interested parties. Finding a common language that will be clearly understood by all parties is needed in explaining the results and the processes of the risk assessment and risk management processes.

In the context of this document, the goals of risk communication are: (1) to improve the knowledge and understanding on all aspects of the risk analysis process by all interested parties concerned with risk; and (2) to promote interactive communication between risk assessors, risk managers and other interested parties concerned with risks in order to achieve the desired outcomes.

Understanding Risk Perception

GMOs are oftentimes controversial because the public have different perceptions of the potential risks that GMOs pose to people and environment. Different perceptions of risk pose present one of the most important challenges to risk communication and must be dealt with.

It must be remembered that in risk communication, perception equals reality i.e. perceived risk is real risk; perceived risk has real consequences. The following theories will help explain what happens to a person's ability to assimilate information when they feel threatened.

Risk = hazard + outrage

A deceptively simple formula developed by Dr. Peter Sandman (www.psandman.com), risk includes both hazard and outrage. Hazard includes the objective, technical and measurable component of risk; outrage includes cultural, emotional, and personal factors, including all levels of fear, anger, and general upset.

Public perception of risk is highly influenced by a large number of outrage or risk perception factors. Risks are perceived either as low or high depending on whether some or all of the outrage factors shown in Table 5 are involved. For example, a high risk hazard that is familiar and voluntarily used may be perceived to be less risky.

In addition to the risk or outrage factors, there are other factors that also contribute to differences in perceptions of risk. These are: (1) the language used to describe the risk i.e. statistical language of scientists, and the intuitive language of the public. For example, in describing the safety of GMO, the public language uses 'safe' when what it means is "as safe as" in the language of a scientist; (2) transparency or access to information; and (3) individual/group values, beliefs and interest (e.g. business, environment).

Trust Determination Theory. People who are upset or feel threatened are often distrustful of others. Trust and credibility are two of the cornerstones of effective risk communications. Without them nobody will listen to your message, people will not make informed decisions and action, and problems can get worse.

Table 5. Risk perception or outrage factors (Source: V.T. Covello. www.centre4riskman.com/downloads/rc_slides_2002.ppt)

Lower perceived risk	Higher perceived risk
Trustworthy sources	Untrustworthy sources
Substantial benefits	Few benefits
Voluntary (personal control)	Involuntary or mandatory (no personal control
Natural origin	Human origin (man made)
Familiar	Unfamiliar
Fair/equitable	Unfair/inequitable
Certain	Uncertain
Moral/ethical	Immoral/unethical
Immediate effects	Delayed effects
Effects reversible	Effect irreversible
Scientifically well understood	Not scientifically well understood
Random/scattered	Catastrophic
Little media attention	More media attention
Victim statistical	Victim identifiable

The factors that build trust and credibility are -

- empathy and caring - 50%
- honesty and openness – 15-20%
- competence and expertise – 15-20%
- dedication and commitment. – 15-20%

In high concern/low trust situations, people want to know that you care before they care what you know. Over 50% of your credibility will depend on whether or not you are caring and compassionate. The higher the level of distrust, the more compassion and sympathy is needed.

Credibility transference. You can build trust and credibility by using support from and working with credible third party (other) sources. The idea is to increase your credibility to the same level as a source with high credibility by enlisting its support to your position on the issue. You can also build up your credibility by getting many highly credible sources to support your position on an issue.

Credibility reversal. In cases of attack on credibility, the source of information with lower credibility end up lowering even more its credibility when it attacks a source with higher credibility. Remember, the only information source that can effectively attack the credibility of another source is one of equal or higher credibility.

Credibility ranking of information sources:

- High: health professionals, scientists, educators, advisory groups
- Medium: Media, activist groups
- Low: Industry, paid external consultants

***Mental Noise Theory*:** When people are upset they have difficulty hearing, understanding, and remembering information. This implies limited attention to information and limited ability to process and retain information.

A highly effective verbally-communicated message should

- limit the number of messages: 3 key messages
- be clear: 6th grade level or 12 year-old language
- be brief or concise: 10 seconds or 27 words
- be repeated 3X: Tell them what your going to tell them. Tell them. Tell them what you told them.
- be assisted by visual graphics, slides
- be aware that it takes three (3) positive messages to balance one (1) negative statement 3N=1P
- not contain negative words: No, Not, Never, Nothing, None

In case of non-verbal message or body language, it is important to remember that body language often overrides verbal communication. It can provide up to 75% of message content. It is noticed intensely and is easily negatively interpreted. For example, poor eye contact can leave an audience feeling that you are dishonest, unconcerned or nervous; or a raised voice can send the message that you are hostile, nervous, or deceitful.

Applying Risk Communication Principles in Risk Analysis

The joint FAO/WHO expert consultation on the application of risk communication to food standards and safety matters identified the elements, principles, barriers and strategies for effective risk communication (FAO, 1999). The principles, applied to risk assessment and risk management processes, are illustrated below:

- *Know the audience.* In the risk analysis process, the different types of audience are: risk assessors, risk managers, government, interest groups and the general public. It is important to listen to and understand their motivations, opinions, concerns and feelings. These are important in the development and delivery of credible information on the risk identified, the decisions made, and the processes used. Understanding the audience's perception of risk can be done through surveys, interviews, focus groups
- *Involve the scientific experts.* Scientific experts are primarily involved in the risk analysis process in their capacity as risk assessors. They work very closely with the risk managers in arriving at the final decision on any dealing with GMO. These experts must be able to explain clearly the results of their assessment including the assumptions and subjective judgments so that risk managers can clearly and fully understand the risks and consequently inform their decision.

- *Establish expertise in communication.* The risk analysis process generates enormous amount of information of interest to a wide range of audience. Developing credible information and delivering them effectively require communication expertise. Risk communication experts have to be involved as early as possible. Communication expertise of risk managers and risk assessors has to be improved by training and experience.
- *Be a credible source of information.* In the risk analysis process, the sources of information are risk assessors, risk managers, applicant, and other interested parties. Information from a credible source will likely be accepted. For example, information of Codex Alimentarius Commission on food safety assessment will more likely be accepted than information from a company consultant. Consistent messages from multiple sources lend more credibility to the risk assessment. Results of safety assessment by regulatory bodies of many counties of a particular GMO will likely be more accepted. To be credible, the source of information should be perceived as genuinely concerned with their views and opinions on the risk issues, trustworthy, competent, committed and consistent. Timeliness in delivery and up-to-date information to address current issues and problems adds to the credibility of the source.
- *Share responsibility:* There are multiple actors involved in the risk analysis process. These include risk assessors, risk managers, other interested parties and the media. Each has a specific role to play, but have joint responsibility for the outcome. Since science must be the primary basis for decision making, all parties involved in the communication process should know the basic principles and data supporting the risk assessment and the policies underlying the resulting risk management decisions.
- *Differentiate between science and value judgment* It is essential to separate "facts" from "values" in reporting the results of the risk assessment and decisions made in the risk management process.
- *Assure transparency.* For the public to accept the risk analysis process and its outcomes, the process must be transparent. This means the process and results of risk assessment and risk management are accessible and available for examination by interested parties, but giving due regard to confidentiality of information (if allowed by regulation)
- *Put the risk in perspective.* In the process of risk analysis, this can be done by emphasizing the information about the risk that are relevant to help the target audience makes up its mind. For example, in the decision-making step, the risk manager may examine the risk in the context of the benefits associated with the technology. Risk comparison that underestimates the concern should be avoided.

Facilitating Public Engagement in the Risk Analysis Process

Risk communications not only aims at informing and educating the public i.e. improving the understanding of risk issues but also at dealing with conflicting views and interest of the regulators, other interested parties and the general public on all aspects of the risk analysis process. Engaging all parties in a responsive and interactive dialogue will may not change their individual positions but will lead to a better understanding of and increased level of acceptance in the decisions made.

The need to engage the public in decision-making processes concerning safety of GMO to people and environment is increasingly being recognized. This trend is clearly presented in the results and background document of the FAO Biotechnology Forum[15]. One of the decision-making processes identified where public engagement is needed is in the risk assessment and risk management, particularly in the approval of GM products. However, there are still no internationally agreed guidelines as to what extent and how public input can be integrated into the risk analysis process.

The joint FAO/WHO expert consultation on application of risk communication to food standards and safety matters (1999) identified steps in the risk analysis process where public input maybe considered. The most important is in the risk management step specifically in the identification and weighting of policy and decision alternatives by risk managers. It was suggested that interested parties, whenever practical and reasonable, should be involved in identifying management options, developing criteria for selecting those options and providing input to the implementation and evaluation strategy.

IDS (2003) also considered some of the choices regarding at which point the public could be involved in decision-making process in the implementation of regulatory framework. In the context of the risk analysis process, some of the choices identified are: (1) identification or risk issues (what do citizens know, what they are concerned about); (2) roles, duties and powers of responsible agencies; (3) mechanisms of reporting, public scrutiny and accountability; (4) location and design of biosafety trials. The kinds of processes that then maybe used include: (1) engaging with areas of public concern (rather than assuming what people need to know); (2) ensuring openness about applications for biosafety review and commercialization; (3) ensuring openness about the purpose, location and design of biosafety trials; (4) ensuring opportunities for public comment. The kinds of tools which maybe considered include stakeholder forums that are accessible and widely advertised and public registers of applications under review, with routine opportunities for public comment and obligations to respond to public comments.

As a final note, IDS emphasized that public participation is highly contextual. While the concerns are similar, there is no ‘one-size-fits-all’ formula for public participation and awareness-raising. What works in some places or in some circumstances will not work

[15] Please see Ruanne J and A Sonnino. 2006. Results from the FAO Biotechnology Forum Background and dialogue on selected issues.

everywhere. Appropriate forms of public participation and consultation need to take into account the different situations, capabilities, and stages of development of each country.

REFERENCES

Websites:

http://oba.od.nih.gov – National Institute of Health, USA

http://www.cdc.gov – Center for Disease Control

http://www.who.org – World Health Orgnaization

http://www.biosafety.be – Biosafety Belgium

http://www.cbd.int – Convention on Biological Diversity

http://www.fao.org – Food and Agriculture Organization

http://pubresreg.org- Public Research and Regulation Initiative

http://www.oecd.org – Organization for Economic Cooperation and Development

Basic Concepts

Belgian Biosafety Server. 2006. International classification schemes for micro-organisms based on their biological risks. URL: http://www.biosafety.be/RA/Class/ClassINT.html

Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. 2005. Advisory Committee on Dangerous Pathogens. Department of Health and Department of Environment, Food and Public Affairs. United Kingdom. Available at: www.hse.gov.uk/aboutus/meetings/committees/acdp/050208/acdp88p6.pdf

Biosafety in Microbiological and Biomedical Laboratories (BMBL). 2007. ***5th Edition***. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention and National Institutes of Health. Washington. Available at: http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL_5th_Edition.pdf

Consulting and Audit Canada for Emergency Preparedness Canada. 1995. Managing Biological Risk with an emphasis on risks associated with biotechnology. URL: http://www.dsp-psd.pwgsc.gc.ca/Collection/D82-54-1995E.pdf

Caucheteux, Danielle and Pierre Mathot. 2005. Biological Risk Assessment: An Explanation Meant for Safety Advisors in Belgium. Applied Biosafety, 10(1) pp. 10-29. Available at: http://www.absa.org/abj/abj/051001caucheteux.pdf

Dreazen, Orna. The application of the OECD principles of GLP-Comparison with ISO/IEC 17025 requirements URL: http://www.ema.org.mx/descargas/especial/dia2/BuenasPracticasLaboratorios.pdf

Env/Mc/Chem(98)17: OECD Series On Principles Of Good Laboratory Practice And Compliance Monitoring Number 1. OECD Principles On Good Laboratory Practice (As Revised In 1997). Paris, France. URL: http://www.oecd.org/ehs

Fox, Arlene. 2003. GLP regulations vs. ISO 17025 requirements: how do they differ? Accred Qual Assur 8:303 DOI 10.1007/s00769-003-0623-y.

Laboratory biosafety manual. 3rd ed. Geneva: World Health Organization; 2004.

NIH Guidelines For Research Involving Recombinant DNA Molecules (NIH Guidelines) April 2002 URL: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

Traynor, Patricia,Dann Adair, Ruth Irwin. Greenhouse Research with Transgenic Plants and Microbes: A Practical Guide to Containment. http://www.isb.vt.edu

Risk Analysis and Risk Management (including socio economics)

UNEP/CBD/BS/COP-MOP/4/INF/22. 2008. Compilation of Guidance Documents on Risk Assessment and Risk Management Available through the Biosafety Information Resource Centre of the Biosafety Clearing-House Conference held on 12-16 May 2008 at Bonn, Germany.

Note: contains 155 documents on risk assessment and risk management currently available *at:* http://www.cbd.int/biosafety/issues/risk.shtml

Andersen, Mark C., Megan Ewald and Jason Northcott. 2004. Risk analysis and management decisions for biological control agents: Perspectives from theoretical ecology. Available at: http://www.usda.gov/oce/reports/risk/AndersenBioControlReport.pdf

Biosafety Regulation: A Review of International Approaches. 2003. The World Bank Agriculture and Rural Development Department.

Cartagena Protocol on Biosafety: Sharing of Peer Reviewed Analyses and of Socio-economics Impacts. 2008. Available at: www.bio.org/foodag/background/FINAL_Socioeconomic_Impacts_MOP-4_outreach.pdf

Cellini, A. Chesson, I. Colquhoun, A. Constable, H. V. Davies, K. H. Engel, A. M. R. Gatehouse, S. Kärenlampi, E. J. Kok, J. -J. Leguay, S. Lehesranta, H. P. J. M. Noteborn, J. Pedersen and M. Smith 2004. Unintended effects and their detection in genetically modified plants. Elsevier Ltd. Food and Chemical Toxicology 42 (2004) 1089-1125. Available at: http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6P-4C004D33&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=02997d8e5098161ab4f9a0ccf3bb81c9

Chassy, Bruce M. 2002. Food Safety Evaluation of Crops Produced through Biotechnology. Journal of the American College of Nutrition, Vol. 21, No. 3, 166S-173S (2002). Published by the American College of Nutrition.

Chen, Mao, Zhao, J-Z, Collins, H.L. Earle, ED, Cao, J. et.al. 2008. A Critical Assessment of the Effects of Bt Transgenic Plants on Parasitoids. PLoS ONE 3(5): e2284. doi.10.1371./journal.pone.0002284. Available at: http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0002284

Codex Alimentarius Commission. 2003. Report of the Twenty-Sixth Session. Rome.30 June - 7 July, 2003. Appendix IV. Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization, Rome. Available at: http://www.fao.org/docrep/006/y4800e/y4800e0o.htm#bm24

Codex Alimentarius Commission. 2004. Foods Derived from Biotechnology. Joint ***FAO/WHO Food*** Standards Programme CODEX Alimentarius Commission. Available at: http://www.fao.org/docrep/007/y5819e/y5819e00.htm

European Commission. Guidance Document for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. 2003. Prepared for the Scientific Steering Committee by the Joint Working Group on Novel Foods and GMOs Composed of members of the Scientific Committee on Plants, Food and Animal Nutrition. Available at: http://www.ec.europa.eu/food/fs/sc/ssc/out327_en.pdf

FAO Food and Nutrition Paper NO.70. 1998. The application of risk communication to food standards and safety matters Report of a Joint FAO/WHO Expert Consultation Rome, 2–6 February 1998. URL: http://www.fao.org/docrep/005/x1271e/X1271E00.htm#TOC

Falck Zepeda, Jose. 2008. Socio-economic Considerations, the Cartagena Protocol for Biosafety and WTO: What are the issues and what is at Stake for developing countries of Article 26.1? Paper presented at 12th ICARB Conference: The Future of Agricultural Biotechnology: Creative Destruction, Adoption or Irrelevance. Ravello, Italy. June 12-14, 2008. Available at: http://www.economia.uniroma2.it/icabr/Public//File/Zepeda_Ravello_14_giugno(1).pdf

Halsey, Mark E. 2006. Integrated Confinement System for Genetically Engineered Plants. Program for Biosafety Systems. St. Louis. Missouri, USA: Donald Danforth Plant Science Center. Available at: http://www.ifpri.org/pbs/pdf/pbsbriefhalsey.pdf

Hayes, Keith. Environmental Risk Assessment for GMO's. CSIRO Marine Division and Biodiversity Sector. Available at: apec.biotec.or.th/pdf/KeithHayes.pdf

Hancock. James F. 2003. A Framework for Assessing the Risk of Transgenic Crops. BioScience Vol. 53 No.5.

Gupta, Kavita, J.L. Karihaloo, and R.K. Khetarpal. 2008. Biosafety Regulations of Asia-Pacific Countries. Asia-Pacific Association of Agricultural Research Institutions Bangkok; Asia-Pacific Consortium on Agricultural Biotechnology, New Delhi, and Food and Agricultural Organization of the United Nations, Rome. P.108 + i-x. Available at: www.apcoab.org/documents/bs_pub.pdf

Jaffe, Gregory. 2008. Establishing National Biosafety Regulatory Systems. Key Outstanding Issues Under the Cartagena Protocol for Biosafety. Program for Biosafety System, Brief No. 12. Available at: www.ifpri.org/pbs/pdf/pbsbrief12.pdf

Konig, A. A. Cockburn, R.W.R. Crevel, E. Debruyne, R. Graftstroem, U. Hammerling, I. Kimberg, I. Knudsen, H.A. Kuiper, A.A.C.M. Peijnenburg, A.H. Penninks, M. Poulsen, M. Schauzu, J.M. Wal. 2004. Assessment of the Safety of Foods Derived from genetically Modified (GM) Crops. Food and Chemical Toxicology 42 (2002) 1047-1088. Elsevier Ltd. Doi:10.1016/j.fct.2004.02.019

Lehrer, Samuel B. Potential Health Risks of Genetically Modified Organisms:

How Can Allergens be Assessed and Minimized? Agricultural Biotechnology and the Poor. Available at: http://www.cgiar.org/biotech/rep0100/Lehrer.pdf

Lemaux, Peggy G. 2008. Genetically engineered Plants and Foods: A Scientist's Analysis of the Issues (Part I). Annu. Rev. Plant Biol. 2008. 59:771-812. Available at: http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.arplant.58.032806.103840

Meek, Sue and Paul Keese. 2006. Putting Theory Into Practice – Applying Risk Analysis to the Regulation of GMOs. Melbourne, Australia. Available at: www.gmo-safety.eu/pdf/biosafenet/Meek.pdf

OECD. An Overview of the Workshop: Beyond the Blue Book – Framework for Risk/Safety Assessment of Transgenic Plants http://www.oecd.org/dataoecd/7/55/39018232.pdf

Peterson Garry, Saul Cunningham, Lisa Deutsch, Jon Erickson, Allyson Quinlan, Ernesto Raez-Luna, Rob Troell, Peter Woodbury and Scot Zens. 2000. The Risks and Benefits of Genetically Modified Crops: A Multidisciplinary Perspective. Conservation Ecology [online] URL: http://www.consecol.org/vol4/iss1/art13/

PRRI Guide on Notifications of Risk Assessment http://pubresreg.org/index.php?option=com_docman&task=cat_view&gid=48&Itemid=58

Rationalizing and Harmonizing Plant Biotechnology Regulations in Southeast Asia: A Learning Forum 4-7 March 2008 Miracle Grand Convention Hotel, Bangkok, Thailand.

Note: contain several powerpoint presentations on status of biosafety regulation of various SEAsian countries, and lectures on environmental risk assessment, plant biotechnology

Available at http://www.searca.org/web/training/courses/2008/march_c/index.html

Risk Analysis Framework. 2005. Department of Health and Ageing. Office of Gene Technology Regulator. Australia Available at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/$FILE/raffinal2.2.pdf

Romeis, Jorg, D. Bartsch, F. Bigler, M.P. Candolfi, M.M.C. Gielkens, S.E. Hartley, R.L. Hellmich, J.E. Huesing, P.U. Jepson, R. Layton, H. Quemada, A. Raybould, R.I. Rose, J. Schiemann, M.K. Sears, A. M. Shelton, J. Sweet, Z. Vaituzis and J.D. Wolt. 2008. Assessment of Risk of Insect-Resistant Transgenic Crops to Non-Target Arthropods. Nature Biotechnology, Volume 26, Number 2.

Ruane, John and Andrea Sonnino. 2006. Results from the FAO Biotechnology Forum: Background and Dialogue on Selected Issues. FAO Research and Technology Paper 11 FAO. Rome, Italy.

Ruane, John and Maria Zimmermann. 2001.Agricultural Biotechnology for Developing Countries: Results of an Electronic Forum. FAO Research and Technology Paper 8. FAO. Rome, Italy.

Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects. 2004. The National Academies Report in Brief.

Secretariat on the Convention of Biological Diversity. 2000. Cartagena Protocol on Biosafety to the Convention on Biological Diversity: text and annexes. Montreal: Secretariat on the Convention of Biological Diversity. Available at: http://www.cbd.int/doc/legal/cartagena-protocol-en.pdf

Spok, Armin, Heinz Hofer, Petra Lehner, Rudolf Valenta, Susanne Stim and Helmut Gaugitsch. 2004. Risk Assessment of GMO Products in the European Union. Toxicity Assessment, Allerginicity Assessment, and Substantial Equivalence in Practice and Proposal for Improvement and Standardization. Umweltbandesamt GmBH Wein, Vienna, Austria. Available at: http://www.umweltbundesamt.at/fileadmin/site/publikationen/BE253.pdf

The Use of Genetically Modified Crops in Developing Countries: A Follow-up Discussion Paper. 2004. Nuffield Council on Bioethics. London.

Wolt, Jeffrey, D. and Robert K.D. Peterson. 2000. Agricultural Biotechnology and Societal Decision-Making: The Role of Risk Analysis. AgBioForum, Volume 3, Number 1, 2000 pp. 39-46. Available at: http://www.agbioforum.org/ v3n1/v3n1a06-wolt.pdf

Risk Communication

Website

http://www.psandman.com

http://www.centre4riskman.com/downloads/rc_slides_2002.ppt)

http://www.astho.org

Association of State and Territorial Health Officials (ASTHO).2002. Communication In Risk Situations. Responding To The Communication Challenges Posed By Bioterrorism And Emerging Infectious Diseases. Available at: http://www.astho.org/pubs/astho%20risk%20communication%20e-workbook.Htm

Communicate Risk. 2001. Meeting of Senior Officials from Centers of Government on Risk Management, Reykjavik, 22-23 October 2001. Organization for Economic Co-operation and Development.

Covello, Vincent 2002. Risk and Crisis Communication. PowerPoint Presentation

Center for Risk Communication. New York.

FAO. 1999. The Application of Risk Communication to Food Standards and Safety Matters. 1998. Report of Joint ***FAO***/WHO Expert Consultation. Rome 2-6 February 1998. Food and Nutrition Paper 70. Availabe at: ftp://ftp.fao.org/docrep/fao/005/x1271e/x1271e00.pdf

Risk Communication Tool. 2003. Electronic Resource and Preservation Network. Available at: http://www.erpanet.org/guidance/docs/ ERPANETRiskTool.pdf

Fischhoff, Baruch. 1995. Risk Perception and Communication Unplugged: Twenty Years of Process. Society for Risk Analysis. Risk Analysis, Vol. 15, No. 2, 1995. Society for Risk Analysis. Available at: http://www.sds.hss.cmu.edu/media/pdfs/fischhoff/FischhoffUnplugged.pd

National Research Council. Improving Risk Communication: Free Executive Summary

Committee on Risk Perception and Communication, Available at: http://www.nap.edu/nap-cgi/execsumm.cgi?ecord_id=1189

Public Participation and the Cartagena Protocol for Biosafety. A review for DfID and UNEF-GEF. Available at: http://www.ids.ac.uk/UserFiles/File/knots_team/NBFreport_Main.pdf

Reeves, Cliona. 2007. Managing Outrage and Crises: Dealing with Risk by Understanding your Audience. Guelph Food Technology Center News reprint. Number 49.

Risk Communication Manual. 2005. Capacity Building in Biosafety of GM Crops in Asia. Food and Agriculture Organization of the United Nations. Bangkok, GCP/RAS/185/JPN. Document No. 1.2005.

Torgersen, Helge. 2004. The Real and perceived Risks of Genetically Modified Organisms. Europe Molecular Biology Organization Reports. Special Issue Vol. 5, 2005, pp. 817-821. Available at: http://www.oeaw.ac.at/ita/ebene5/HT_1243.pdf

Zepeda, Cristobal. 2004. Risk Communication. OIE Conference. Available at: http://www.oie.int/downld/Panama_riskcom_nov04.pdf