The proposed Biosafety Act of Sri Lanka ; Environmental Risk Assessment ensures the safe use of GMOs

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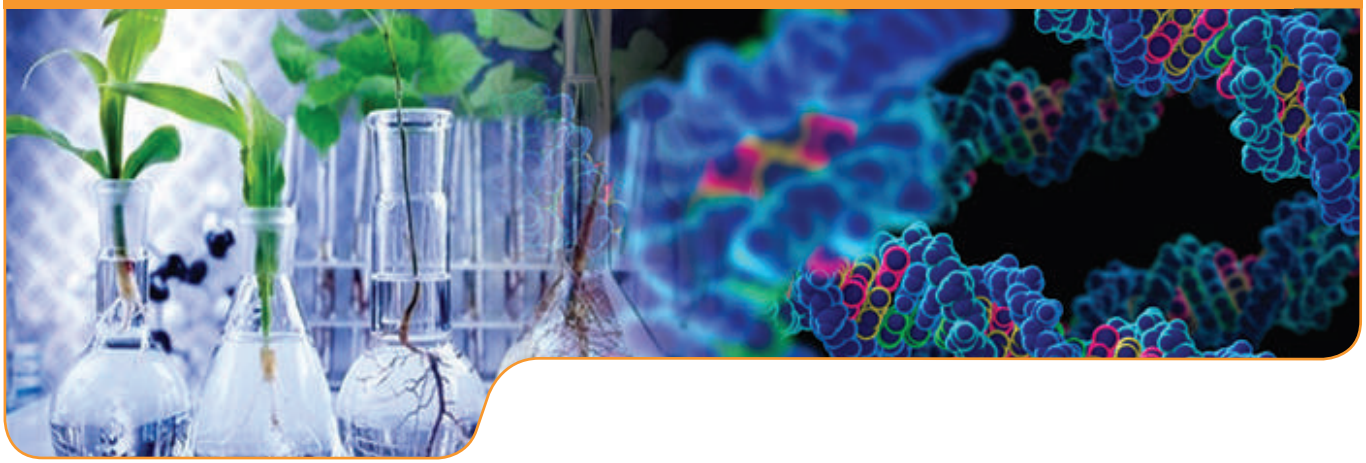


Biosafety, in this context, refers to Genetically Modified Organisms, Food, Feed and Processed products (GMO / FFPs). The GMOs (also known as Living Modified Organisms, Transgenic Organisms) are produced by using the modern biotechnological tool, recombinant DNA technology (rDNA technology % genetic engineering). In this procedure, genes can be isolated, cloned and transferred to the DNA of other unrelated organisms, i.e. genes can be transferred across species and even across Kingdoms. Hence, a GMO will carry a new gene that produces a new protein for a new character that the target organisms did not possess in its natural state.

Characters, Genes, Genomes

Every living being is described by using characters. How do characters appear? Characters appear due to the expression of genes. If we consider a single hair, it has several characters such as colour, thickness and shape. Colour appears due to the expression of a particular gene, which produces a protein that gives colour to the hair. Minor differences in the same gene gives different hair colours such as black, blond, brunet etc. Another gene produces the shape such as wavy hair. Minor differences in the same gene produces straight hair or curly hair. The gene for thickness acts in the same way. Hence, in order to produce all the characters of a human being, how many genes would be necessary? Up to date, around 40,000 genes have been identified.

What are genes and where are they?

A gene is a part of the DNA Deoxyribo Nucleic Acid molecule that resides in the nucleus of every

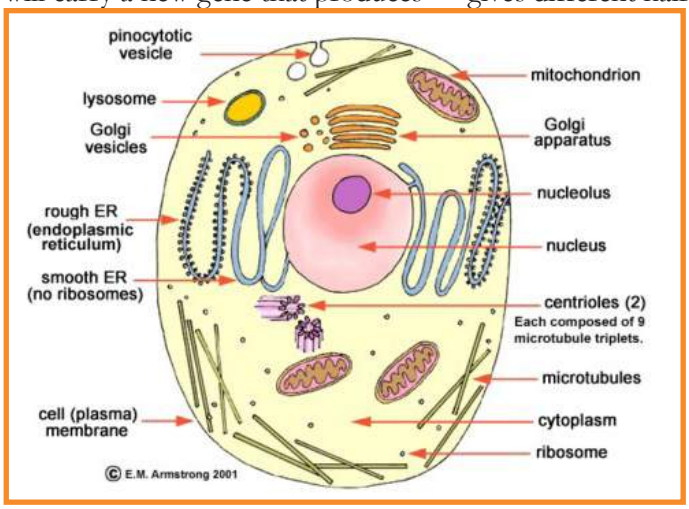


Fig. 1 : The components of a cell. Every cell has the nuclear material (DNA)

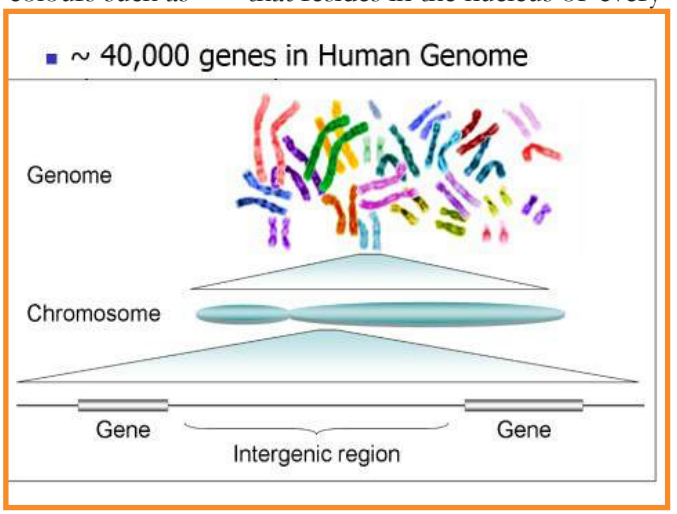


Fig. 2 : The 23 pairs of chromosomes = 46 chromosomes in a human cell
Genome = Total amount of DNA in the 23 chromosomes

cell (Fig.1). DNA is known as the nuclear material. It is a chemical, an acid and hence each DNA molecule is wrapped around a protein for stability. This structure is called a chromosome. A human cell has 46 chromosomes i.e. 46 DNA molecules (Fig.2). Of these, 23 come from the mother and the other 23 from the father. The total amount of DNA in a set of 23 chromosomes is known as the Genome.

The DNA molecule

The fundamental building block of DNA is the Nucleotide (Fig. 3).

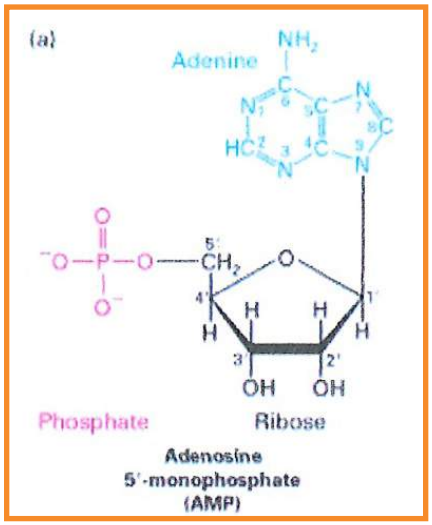


Fig. 3 : A nucleotide carrying a ribose sugar, phosphate and the base Adenine

A single nucleotide is made of a sugar entity (Ribose), a Phosphate and a base. Each nucleotide will carry one of the four types of bases Adenine (A) Guanine (G), Cytosine (C) or Thymine (T). The difference between nucleotides is the type of base that each will carry.

Nucleotides join together by strong bonds (phospho-diester bonds) to form a single strand. Two such strands join together by weak bonds (hydrogen bonds) to form a single DNA molecule (Fig. 4). As it is very long, it takes the form of a helical structure in order to fit into the nucleus (Fig.5).

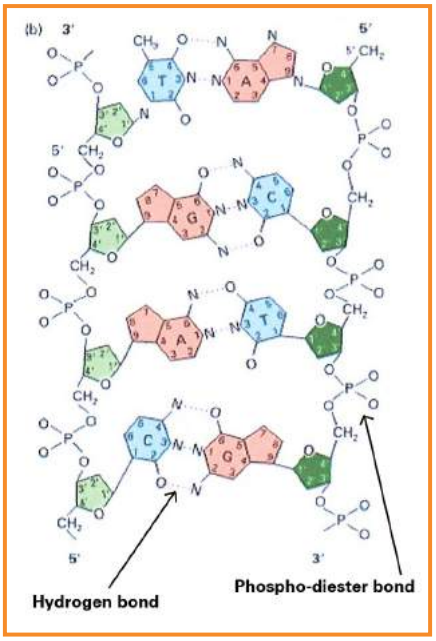


Fig. 4 : Joining of two strands forming the DNA molecule

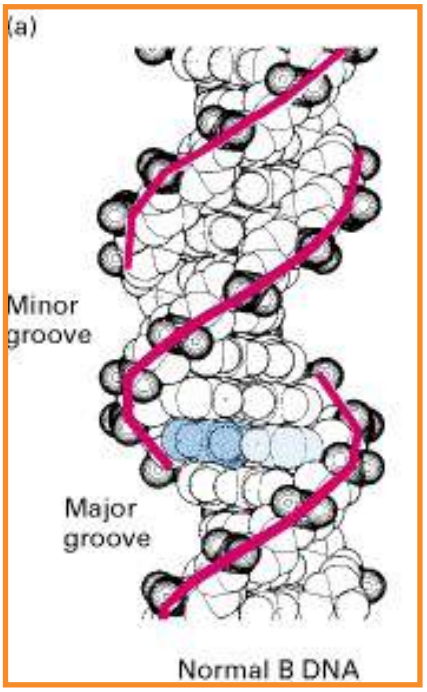


Fig. 5 : Twisting of the two strands forming the DNA double helix

A gene is made up of a sequence of these nucleotides joined together in a single strand and is denoted as the particular sequence of bases it is made up of, such as ……CCTGGCTGGAATC…. and so on, giving a message to produce a particular protein. Different genes have different sequences, thus producing different proteins for different characters. However, genes make up less than 10% of the genome.

Regulation of gene expression.

Are all the 40,000 genes in our cells functioning at the same time? No, of course not ! Some genes that were expressed when we were in our mothers' wombs are 'switched off' now and some that were 'switched off' then are 'switched on' now. A single cell in our heart will also carry the gene for hair colour, but it is 'switched off' from the beginning of life. Therefore,

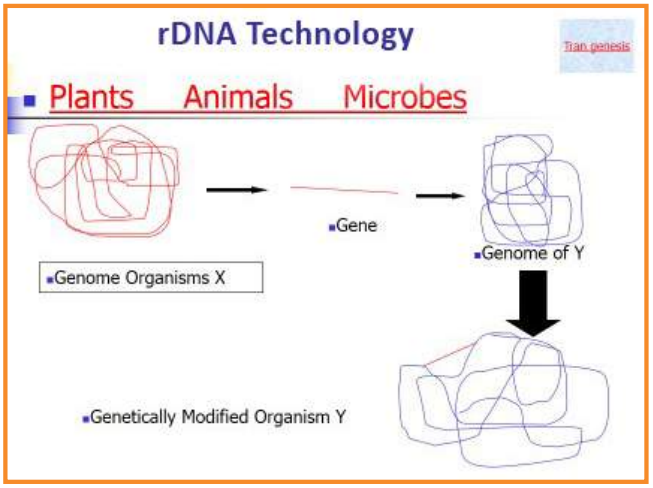


Fig. 6 : A simplification of the technology

we see that gene expression is regulated, and controlled. This is carried out by another fragment of DNA known as the Promoter. Hence, for a gene to be expressed, a promoter sequence is also necessary.

Recombinant DNA technology

It is now possible to identify and isolate any gene of interest from a known genome, clone it in a vector so as to multiply it, and then transfer it to a genome of another organism (Fig.6). The most commonly used techniques for gene transfer are the *Agrobacterium*-mediated gene transfer method and the use of the Gene Gun. Organisms produced from such a technology are known as Genetically Modified Organisms (GMOs). Any food or animal feed obtained from them are known as Genetically Modified Food and Genetically Modified Feed respectively, while processed products may also carry GM ingredients in them. All of these are designated as GMO/FFPs.

Products of GM technology

This includes plants, animals, insects and microbes.

Examples of GM plants

Some examples of GM plants grown extensively include Biotech Corn, Bt Cotton – Corn and Cotton plants carrying a bacterial gene conferring resistance to a particular Lepidopteran insect pest; Herbicide tolerant Soyabean – plant carrying a bacterial gene conferring resistance to a particular herbicide.

Some other GM plants include Flavr-Savr tomatoes, Virus resistant Papaya, Bt Brinjal.

Global use of GMOs

The global use of GMOs is shown in Figure 7.

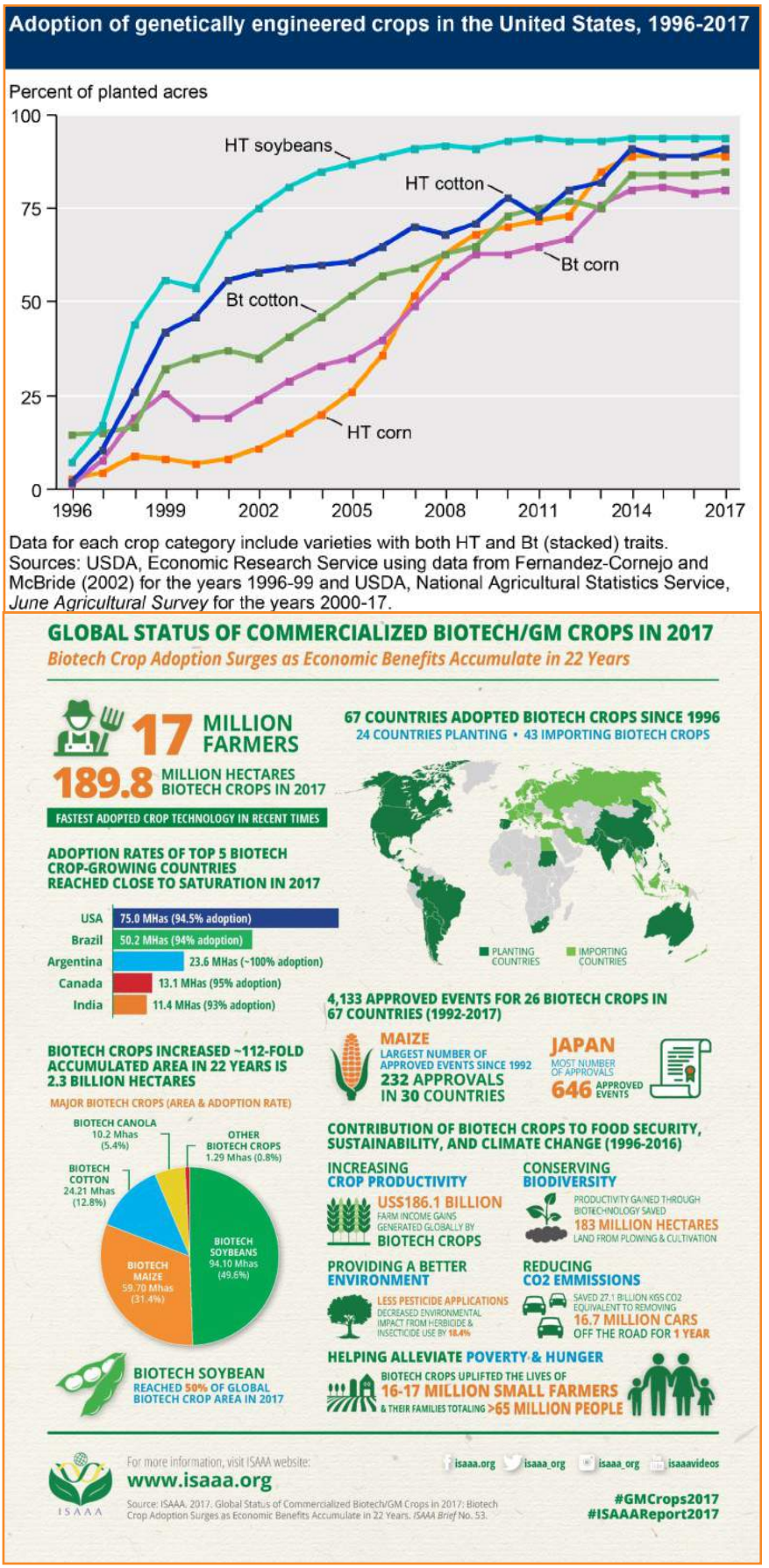


Fig. 7 : Global use of GMOs

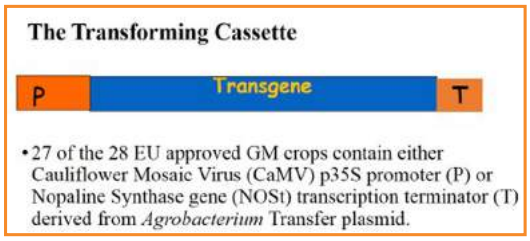


Fig. 8 : An example of a basic DNA cassette used for transformation

The Transforming Cassette

A DNA cassette is a DNA construct that carries the DNA parts that would be 'cut' and transferred to the recipient genome (Fig.8 & Fig.9).

Fundamentals of molecular biology reveal that a gene by itself cannot function or express itself to produce a protein. It is regulated by another fragment of DNA known as the Promoter that has the ability to 'switch' a gene ON or OFF. Hence, when transferring a gene to another genome, a Promoter sequence too must be included. Most GM plants contain the strong promoter (CaMV)p35S obtained from a microbe, in order to 'power' the maximum production of the protein. Could this promoter pose any risks?

In the production process of a GM plant, there are two stages that require selection –

(i) Selection of the vector that carries the transforming cassette from those that do not contain it. This requires a Marker gene, usually an antibiotic resistant gene and its promoter to be included in the cassette

(ii) Selection of the plant cells in which the cassette has been successfully inserted into the genome. Some cells may not take up the cassette. This requires another Marker gene or Reporter gene and its promoter to be included in the cassette.

The cassette also requires the insertion of a terminator sequence to denote the end of the gene sequence.

Possible Risks of GMO/ FFPs to human health and the environment

In observing the above details of the technology, scientists have acknowledged the possibility of this technology posing risks to the environment and human health, which should be considered before permitting the use of GMO/FFPs. This is indicated in the Convention on Biological Diversity, through which the Cartagena Protocol on Biosafety was established.

The Convention on Biological Diversity (CBD)

The CBD was inspired by the world community's growing commitment to sustainable development. It emphasizes the importance of conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefit arising from the use of genetic resources as many indigenous and local communities have a close and traditional dependence on biological resources. The CBD encourages the use of Modern Biotechnology (rDNA Technology) in this process. The CBD entered into force in December, 1993. Sri Lanka has signed and ratified the CBD.

Article 8 (g) of the CBD states thus:

Establish or maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms (includes GMOs)

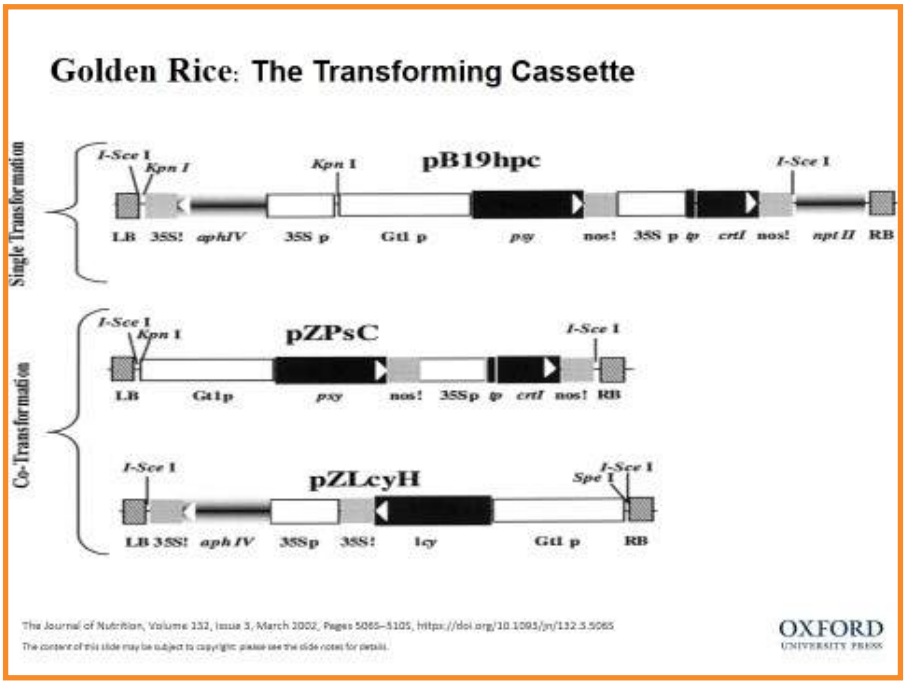


Fig. 9 : The transformation cassette used in the production of Golden rice

resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health.

Article 19, paragraph 3 proposes the need of an International Protocol setting out appropriate procedures to establish safety in the transfer, handling and use of LMOs / GMOs

The Cartagena Protocol (CP) on Biosafety

The result of this was the establishment of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. It was adopted in January, 2000. Sri Lanka signed and ratified it.

The Protocol creates an enabling environment for the environmentally sound application of biotechnology, making it possible to derive maximum benefit from the potential that biotechnology has to offer, while minimizing the possible risks to the environment and to human health, and specifically focusing on transboundary movements of the GMOs.

The CP is based on the Precautionary Principle [Article 11 (8)], which states thus:

" Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a Living Modified Organisms on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of that living modified organism intended for direct use as food or feed, or for processing, in order to avoid or minimize such potential adverse effects."

Assessment of possible risks before releasing a GM plant to the environment, is an essential part of the CP.

Global Restrictions of GMOs

Due to the possible risks involved, many countries and regions have enacted restrictions regarding the movement and cultivation of GMOs.

(i) Cultivation banned, imports banned

Algeria, Bhutan, Kenya, Kyrgyzstan, Madagascar, Peru, Russia, Venezuela, Zimbabwe

(ii) Cultivation prohibited, imports (mostly animal feed) allowed

Austria, Azerbaijan, Belize, Bosnia, Bulgaria, Croatia, Cyprus, Denmark, Ecuador, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxemburg, Malta, Moldova, Netherlands, Northern Ireland, Scotland, Wales, Norway, Poland, Saudi Arabia, Serbia, Switzerland, Turkey, Ukraine

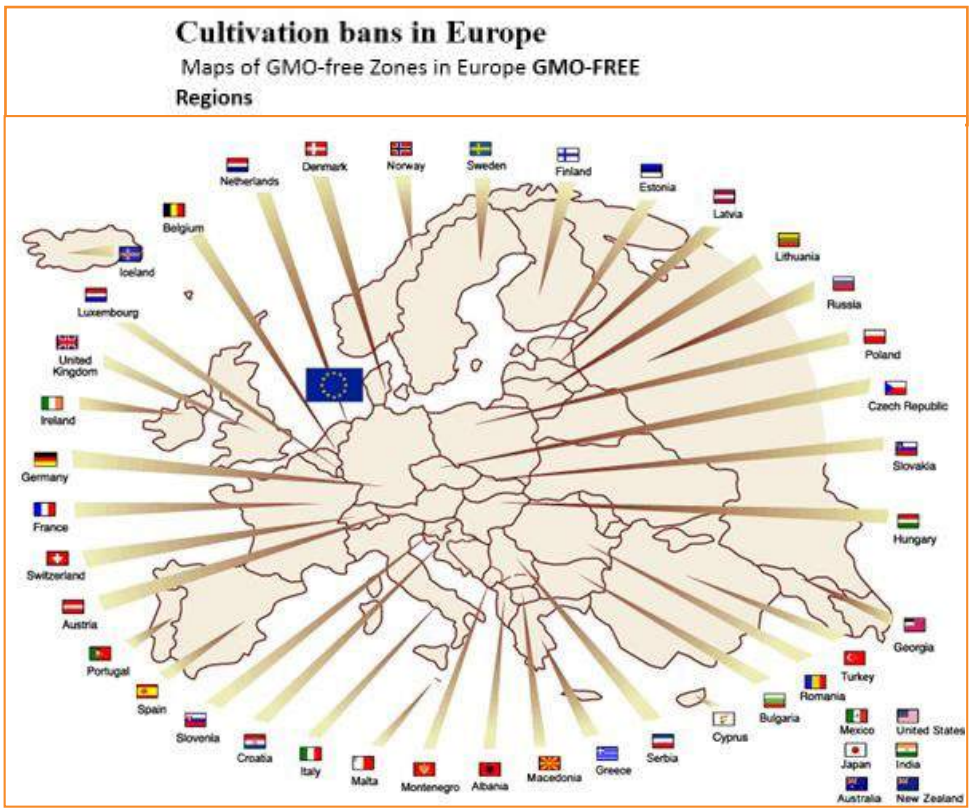


Fig. 10 : Map of GMO-free zones in Europe

(iii)GMO prohibited Regions

California, USA : Cultivation banned, imports allowed
Humboldt + Arcata city
Marin
Mendocino + Point Arena city
Trinity
Santa Cruz
Colorado, Boulder County, USA : planned ban of GM corn and GM sugar beet
Maine, USA
San Juan, Washington, USA
South Australia
Tasmania
Wallonian region, Belgium

The GMO-free zones in Europe are shown in Figure 10.

- Peru has extended its GMO moratorium and Mexico phases out glyphosate and GM maize in food and disallows GM maize releases (2021)

Environmental Risk Assessment

Important risks that arise due to introduction of GMOs to the environment are given below. They are the targets of the hazard/s.

(i) Effects of GMO on biological diversity / centres of origin and diversity
(ii) Movement of transgene to close relatives
(iii) Movement of transgene to non-GM varieties - contamination
(iv) Effect on non-target organisms, including pollinators and natural enemies
(v) Effects on soil organisms
(vi) Evolving resistance to the new protein
(vii) Arising of secondary pests
(viii) Creation of 'super weeds'
(ix) Introgression of the transgene in the population

Risk Assessment

Risk assessment is the core of biosafety, as it represents the science-based approach for decision making towards protection of human health and the environment when dealing with GMOs. The purpose of the risk assessment is to identify, characterize and evaluate potential risks.

A risk arises due to the function of the hazard, the exposure of the target to the hazard, and the consequences due to the exposure.

Risk *f* Hazard x Exposure x Consequences

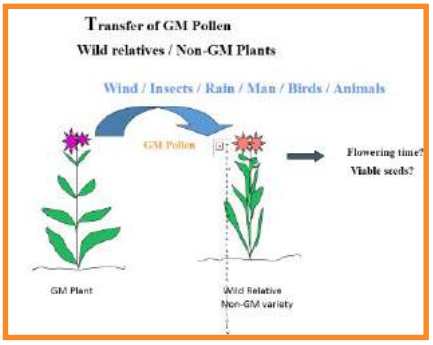
Risks to the Environment

Major Environmental Risks with regard to the release of GMOs to the environment are as follows.

1. Transfer of transgene to wild relatives / Non-GM variety through hybridization

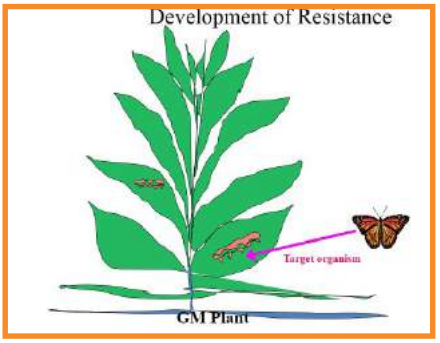
Can a GM plant hybridize with and cause the transfer of the transgene to a wild relative and/or to a non-GM variety through natural pollination?

The hazard in this case is the new gene present in the pollen of the LMO. The exposure of this pollen to the stigma of a wild relative / non-GM variety depends on many factors such as the cultivation distance between the two, the synchronization of flowering, the method of pollen transfer, distance such pollen can travel, pollinators present and the fertility of the resultant seed. The consequences of such an event can then be estimated and a risk assessment made.



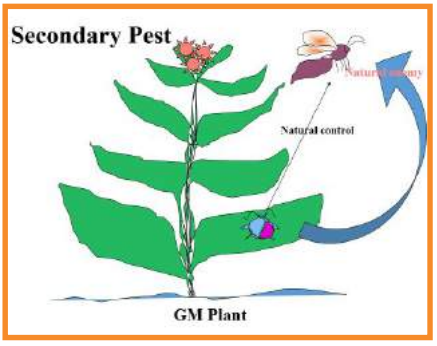
2. Development of resistance

The continuous cultivation of an insect resistant GM variety can, with time, cause the pest/insect to acquire resistance. It has been reported to have happened already. The hazard here is the new protein. The exposure will depend on the continuity of cultivation of the GM variety in the same field or area. In order to manage such a risk, mitigating factors need to be included in the management procedures such as establishment of non-GM refuges / buffer areas for the insects to feed on.



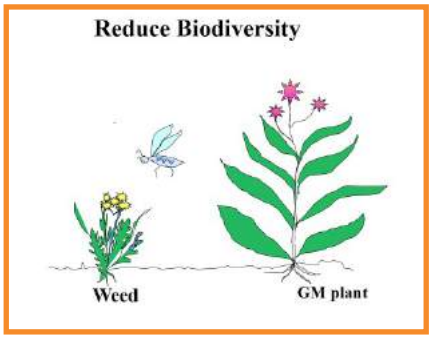
3. Emergence of secondary pests

A pest that the target organism usually controls in the natural environment may become a secondary pest due to the elimination of its predator, the target organism. Here, the hazard is the new protein



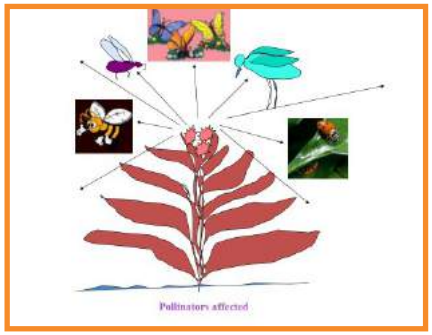
4. Reduction of biodiversity

This can occur, especially in the cultivation of herbicide tolerant GM varieties, where a total eradication of weeds will cause loss of farmland biodiversity due to reduction in food for beneficial insects, birds, other non-target organisms etc. The hazard here is the new protein and the target is the populations of weeds. The consequences will be the loss of biodiversity.



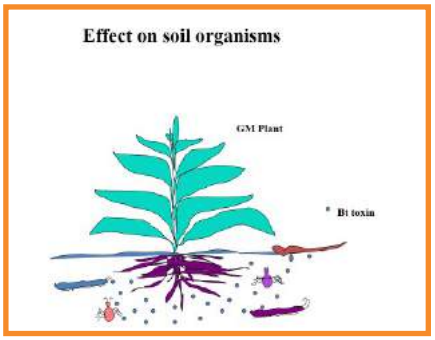
5. Effect on non-target organisms such as pollinators

The hazard here is the new protein. The exposure of this protein to non-target organisms such as butterflies, bees, moths, beetles, birds etc. needs to be estimated. Consequences will be only if the new protein becomes a toxin to such organisms.



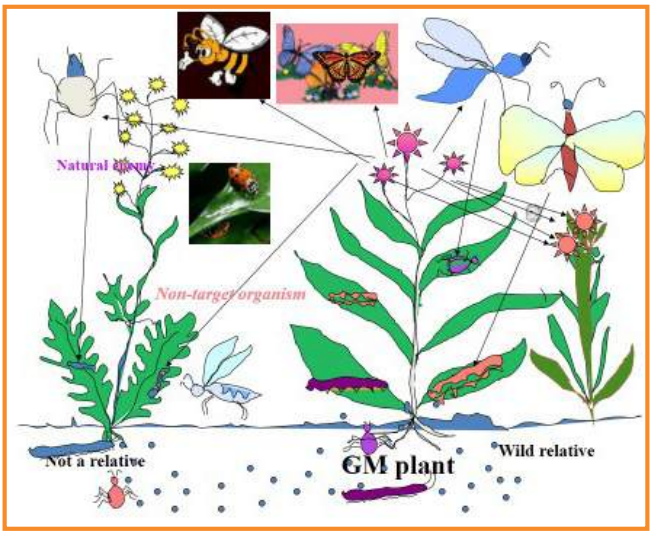
6. Effect on soil organisms

The hazard, which is the new protein can accumulate in the soil from fallen plant parts such as leaves, fruits etc. and harvested plants remaining in the field. A large number of soil organisms may be exposed to this new protein of the GM plant. The consequences would be the effect on the organisms and the resultant effects on soil quality for future cultivations.



7. Complex situation in agricultural fields

The cultivation of a GM plant, therefore, creates a complex set of possible risks. The new gene as well as the new protein are the major, possible hazards. Many targets exist in an agricultural field, each of which needs to be examined. The entire complex scenario must be considered in RA, RM & RC



Risk Analysis Methodology

Risk *f* Hazard x Exposure x Consequences

Risk Analysis of a GM plant (or any other GMO) involves the following steps.

1. Identification of the protection goal.
What are we trying to protect ? In this case it is the environment
(In the case of a GM food it is human health)

2. Identification and characterization of the hazard/s
In this case, we have to identify the hazard/s that could pose a risk.
In a GM plant, the possible hazards are:
The new gene
The new protein
Promoter sequences
Marker genes
Reporter genes.
Other DNA fragments in the cassette

3. For each hazard, we have to identify the target or end-point of the hazard.

E.g. If the hazard is the new gene, then a possible target would be a non-GM variety, where there is a possibility of the new gene contaminating the non-GM variety by moving to it through pollen.

4. For each end point per hazard, estimate the exposure and the consequences by using the RA Matrix (Fig.11).

E.g. What would the chances be of GM pollen contaminating a non-GM variety? What would be the consequences if it would?

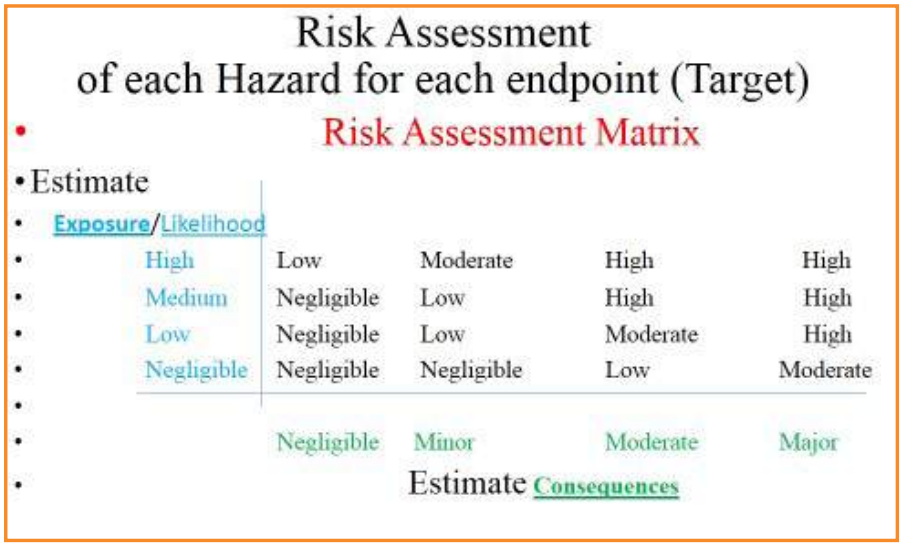


Fig. 11 : The Risk Assessment Matrix

5. Assess the risk for each hazard per each target end-point

Risk assessment *f* estimate of the exposure x estimate of the Consequences

6. Assess the overall risk for the GM orgnism, considering all the hazards and target of each hazard. and provide the Risk Management and Risk Communication procedures.

This information will be sent to the National Competent Authority to make the final decision regarding the release of this GMO to the environment.

As per the Cartagena Protocol, the proposed Biosafety Act of Sri Lanka has established the administrative structure in order to assess the above risks, as shown in Figure 12.

Every application to release a GMO to the environment, made to the National Focal Point / National Competent Authority (NCA) will be sent to the appropriate Sectoral Competent Authority (SCA) in order to carry out a Risk Assessment and report back to the NCA.

In the case of releasing a GM plant to the environment, the SCA will be the Department of Agriculture, who will conduct the Risk Assessment.

The proposed Biosafety Act, will therefore ensure that a scientific risk assessment is carried out before releasing any GMO to the environment.

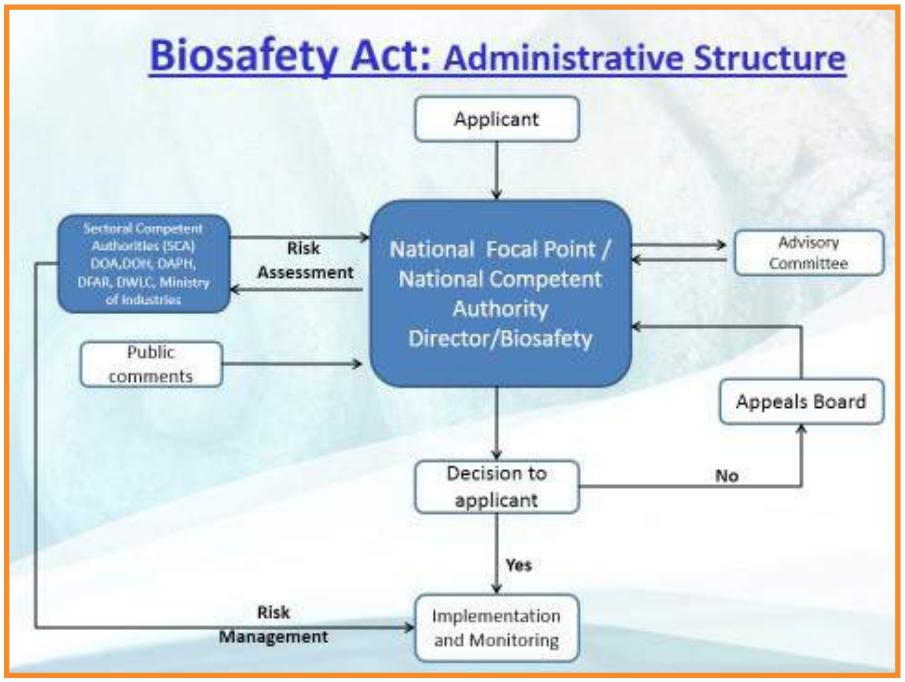


Fig. 12 : The administrative structure for risk assessment

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