



GM Crops and Plant Tissue Culture for Sustainable Agriculture and Health

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The present paper deals with the role of plant tissue culture in production of genetically modified crops. Tissue culture and plant regeneration are integral parts of most plant transformation strategies. For integrating tissue culture and plant transformation strategies a quick and efficient regeneration system must be developed. Selection of the suitable explant, manipulation of different inorganic and organic compounds, growth regulators, growth additives in the nutrient medium are very important to establish the plant in tissue culture system.

Eleusine coracana Gaertn. has high potentiality to regenerate directly from mesocotyl explant in media containing different type of auxins (NAA, IAA, IBA) and growth additive (CM), either alone or in combination. In this case mesocotyl is the best explant for green embryogenic callus. Nodulated callus with heart shaped embryoids were often observed when the culture media was supplemented with slightly higher concentration of 2,4-D. Similarly *Bacopa monnieri*, *Centella asiatica*, *Gynema sylvestris* all medicinally important plants regenerate very well in in-vitro condition. Once the plant is able to establish in Tissue culture system, it can be genetically modified (GM).

Agrobacterium mediated gene transfer and direct gene transfer are widely used methods for transformation. Agrobacterium mediated gene transfer with binary vectors has been successfully applied to a wide range of crop plants. In this technique 'desired gene' incorporates with host genome and after number of divisions it expresses itself.

Four major GM crops, corn, soya, rape-seed and cotton are grown in US, Canada, Mexico, Brazil, Argentina, China, South Africa since 2001. US is the largest producer of GM food crops with 20 million hectare devoted to it.

Keywords: GM crops, Tissue culture, Agriculture, Health.

INTRODUCTION

The genetic modification of crops is one of the most hotly debated current issue. The Scientists (Genetic Engineers) involved in this biotechnological discipline claim higher yield by use of lesser agrochemicals, so are expected to solve problems of world hunger, malnutrition and agrochemicals lead environmental pollution.

Critics of this technology argue, that newly created GM (Genetically Modified) species with new gene combinations could behave in unpredictable and uncontrolled manner, threatening survival of existing species and may impact food safety and human health. (OECD, 1986, Harpenden and Broer, 1996, Jones, 1999, Poppy, 2000, FAO/WHO, 2000).

Genetic modification or engineering enables scientists to create plants, animals and micro-organisms by manipulating genes, inserting genes from one species into the DNA of another species. Such a transfer may even include insertion of animal genes into plants/microbes or vice versa. Genes may also be transferred among species that never normally breed together. The process therefore can create totally new life forms, that never existed on the globe before.

Genetic modification in plants can be considered simply an extension of conventional plant breeding technique. Both are technologies planned for crop improvement in respect to yield potential, quality, harvesting period and resistance against biotic and abiotic stresses.

Plant breeding allows the transfer of genetic information in a precise way through transfer of pollen for introduction of desired traits. This technique has helped a lot in improvement of number of varieties including cereals, pulses, oil seeds, fruits and even ornamental crops in the past 50 years and yet is one of the significant research line. Contributions of different CSIR, ICAR, IARI, NBPGR centers established in almost all states of the country are praiseworthy. In contrast to Genetic engineering, plant breeding is a time consuming process (years are required for creating a new variety through hybridization and selection) and has its limitation of relations. During plant breeding whole set of traits of donor are supposed to be transferred along with pollen grains whereas genetic engineering is a fast avenue, can introduce only desired genes and there is no limitations of relations.

GM plants can be produced by inserting one or more genes artificially from same or quite different species by a process other than pollination. For example Bt. Corn which produces its own insecticide, contains a gene from a bacterium (*Bacillus thuringiensis*). The insertion is done to produce some beneficial properties which are not always agronomic. (Hails, 2000)

Genetic engineering plan is brought in to practice by various tools such as restriction endonucleases (Scissors), vectors, vehicles and a set of *In vitro* conditions for cloning, differentiation and regeneration of plants.

AGRICULTURE AND PLANT TISSUE CULTURE

Plant Tissue Culture is a promising technology in agriculture based on totipotency (plant cells can express total genetic potential, by correct stimuli) and ultimately can develop into whole plant.

Genetic manipulation involves inserting foreign genes or modifying the activity of existing genes. Methods to insert foreign genes are coupled with the methods of Plant tissue culture to regenerate identical populations of plants with novel characteristics. Therefore a quick and efficient regeneration capacity in tissue culture system must be developed prior to undertake genetic manipulation of the cell/plant.

Protocols have been standardized for a number of plant species by different Micropropagation Laboratories set up in India (TERI, NCL) as well as Abroad. Many of these laboratories are exploiting this technique

for clonal propagation with a target of million plantlets for each crop. In our laboratory, trials have been made to regenerate a few important plants viz. *Eleusine coracana*, *Bacopa monnieri*, *Centella asiatica* and *Gymnema sylvestris*, *Mucuna pruriens*.

Eleusine coracana Gaertn. has high potentiality to regenerate directly from mesocotyl explant in media containing different type of auxins (NAA, IAA, IBA) and growth additive (Coconut Milk), either alone or in combination. In this case mesocotyl is the best explant for green embryogenic callus. Nodulated callus with heart shaped embryoids were often observed when the culture media was supplemented with slightly higher concentration of 2,4-D. (Mehta, *et al.*, 1998a, b). Similarly *Bacopa monnieri*, *Centella asiatica*, *Gymnema sylvestris* all medicinally important plants regenerate very well in In-Vitro condition in MS media. In vivo grown stems of *Gymnema* appeared to be the best explant for callusing when media was supplemented with 2, 4-D (2.5 mg/l) or NAA (2.5 mg/l). When leaf segments were inoculated in media with NAA large number of roots with green calli were also recorded.

Complete plantlet formation was seen when field grown part of the stem of *Bacopa* was cultured in combination of IAA (2.5 mg/l) and KN (1 mg/l).

Low concentration of 2, 4-D (5 mg/l) and slightly higher concentration of NAA (2.5 mg/l) were suitable for direct plant regeneration in *Centella*. Leaf segment, nodal parts and young inflorescence of this plant have great potentiality for callusing as well as direct regeneration. Once the plant is able to establish in Tissue culture system, it can be genetically modified (GM).

PLANT TISSUE CULTURE AND GENETIC ENGINEERING

Plant Tissue culture and plant regeneration are integral parts of most plant transformation strategies. For integrating tissue culture and plant transformation strategies a quick and efficient regeneration system must be developed. Selection of the suitable explant, manipulation of different inorganic and organic compounds, growth regulators, growth additives in the nutrient medium are very important to establish the plant in tissue culture system.

A major obstacle in feeding isolated gene directly into the protoplast, cell or tissue, is the presence of so many enzymes that degrade the

foreign DNA. So exogenous DNA needs a good protection so that it can make a safe journey from external medium to the recipient host nucleus so that it can stabilize itself in the recipient's body. Once the plant is able to establish in Tissue culture system, it can be genetically modified (GM).

There are 'molecular scalpel' (Restriction endonucleases) and 'molecular adhesive' (Ligases) to cut and join the particular gene. A vector carrying 'desired gene' is needed which makes the safe journey of exogenous DNA to the recipient host nucleus. *In-Vitro* cloning, differentiation and regeneration of plants are followed.

WHAT ARE GM CROPS

Plants having foreign or altered genes are termed as Genetically Modified (GM) or manipulated or simply transgenic plants. GM crops are scientifically created new life forms that have never before occurred in nature.

There are currently two main types of genetically manipulated crops: those engineered to be tolerant to synthetic herbicides, (Firbank, 1999, 2001) so that farmers can spray their field to eliminate weeds without damaging the crops and those engineered to produce their own toxins that kill pests or diseases that try to attack the crop or interfere with their growth.

NEED OF GM CROPS

The world's population is increasing fastly and is expected to cross 9.5 billion by 2100. Population growth rate is higher in countries with low income where poverty and hunger are widespread. A major sector of people are deprived of food, therefore suffer from malnutrition. The reasons behind such a scenario is the diversion of prime agricultural land to non-agricultural uses to meet growing demand of housing, urbanization and industrialisation. There is desperate need to produce more food from less land with limited water and reduced agrochemical inputs (MAFF, 1998, The Royal Society 1998, 2000).

Second important reason, why we need GM crops is that plants are subjected to predation by virtually all other types of organisms viz. viruses, bacteria, fungi, insects, animals and even weeds. Most conventional insecticides eg. Pyrethroids and organophosphates are relatively non-specific poisons that kill a broad spectrum of insects, not just the ones eating or damaging the crop.. These agrochemicals are costly and due toxic nature

may behave as carcinogens and thus lead to many genetical disorders in the consumers. So current approach is therefore aimed to engineer the crops having potentiality to synthesize their own toxins to develop auto resistance against pests. (Schuler, 1998, Gebhard & Smalla, 1999, Dewar, 2000) Other crop traits such as disease resistance, abiotic stress tolerance and improvement of yield and quality are also considered by genetic engineers. (Scott & Wilkinson, 1999)

GM crops with traits such as increased nutrition, increased yield potential along with increased resistance against various environmental stresses such as drought or salinity might successfully improve agriculture in these developing countries and boost the economy and well being of many people (Oxfam, 1998, Broer, 1996, Beringer, 2000, Christian, 2000, IIASA, 2000).

HOW GENES ARE INTRODUCED

METHODS OF GENE TRANSFER ARE OF TWO TYPES

- A) Direct gene transfer Method including particle bombardment, polyethylene glycol mediated gene transfer, Electroporation etc.
- B) Agrobacterium Mediated Gene transfer.

Agrobacterium mediated gene transfer with binary vectors has been successfully applied to a wide range of crop plants. In this technique 'desired gene' incorporates with host genome and expresses itself.

Agrobacterium tumifaciens is described as nature's smallest Genetic engineer. It is a soil born, gram negative bacterium and is the casual agent of 'Crown gall' disease of many plants (Particularly dicots). This bacterium can be employed for transfer of Recombinant DNA into plant cells, as the bacterium invades plants at the site of wound, transform the cells and cause crown gall. This happens because of Ti plasmid of *Agrobacterium tumifaciens* carries a segment called T DNA which is transferred to plant cell nucleus and becomes integrated with plant's chromosome. This T DNA encodes enzymes that convert plant metabolites into plant growth hormones, auxins, cytokinins on the one hand that stimulate growth and result in gall (tumour) formation and second a series of unusual amino acids the opines which becomes food source for Bacteria.

The integration of T DNA into plant chromosome provides vehicle necessary to introduce new gene into the plant. The transfer of T DNA requires two 25 base pair repeats present on both sides as RB (right border) and LB (Left Border) and also several virulence (*vir*) genes located else where on Ti plasmid. This T DNA region can be harnessed to transfer Recombinant DNA.

TWO PLASMID STRATEGY TO CREATE A RECOMBINANT PLANT

A common cloning strategy employs an Agrobacterium that contains two different recombinant plasmids. The first is a Ti plasmid from which the T-DNA segment has been removed (having only *vir* gene). The second is an Agrobacterium—*E. coli* shuttle vector that contains the T-DNA 25 base pair repeats flanking the gene that a researcher wants to introduce and a selectable marker (an antibiotic resistant gene).

This engineered Agrobacterium is used to infect a leaf at the sites of a wound (the edge of a cut leaf). Crown galls are not formed because genes for auxins, cytokinins and opines have been removed from both plasmids. But the *vir* genes on 1st plasmid mediates invasion and ultimately gene in T DNA region of 2nd plasmid flanked by 25 base pair repeats is transferred to the host genome. New plants are generated when the leaf segments (with transformed cells) are placed on agar dish with controlled level of plant growth hormones and kanamycin. New plantlets are regenerated, which carry the 'desired gene'. Non-transformed plantlets are killed by kanamycin.

A NOVEL METHOD

1. Identify a suitable explant
2. Co-cultivate with the Agrobacterium
3. Kill the bacterium with suitable antibiotic (which does not harm the plant tissue)
4. Select for transformed plant cells
5. Regenerate whole plant.

Dicot plants are easily transformed using standard vectors and standard strain of Agrobacterium such as LBA4404. But cereals (which are not naturally infected by Agrobacterium) require the use of modified vectors or so-called Supervirulent strains of Agrobacterium such as EHA101 or EHA1005. These supervirulence system rely on extra copies of some of the virulence genes either being present on the vector itself or on a separate plasmid in Agrobacterium.

The pioneer work in this field included successful transfer of luciferous genes from firefly into Tobacco plant. Although tobacco is not a very beneficial plant, needing improvement but it is often used experimentally because it is particularly easier to transform with *Agrobacterium*. The potential of this technology is not limited to the production of glow (light) in plants but potential benefits to be drawn by genetic engineering include mainly increase in yield and a reduction in the need of environmentally harmful agricultural chemicals which may lead to severe genetic hazards in the consumers. The same approach has been used to produce resistance for insect pests by means of introduction of Bt genes from the bacterium *Bacillus thuringiensis*.

Genetically modified Tomato plant contains a gene for a protein toxin Cry-A from *Bacillus thuringiensis* confers insect resistance to the plant. This plant shows luxuriant growth in comparison to control plant when exposed to insect larvae under same set of conditions.

INSECT-RESISTANT CROPS

The soil bacterium *Bacillus thuringiensis* (*Bt*) produces Insecticidal Crystal protein(ICP) called δ -endotoxin produced during sporulation of the organism. ICP is an inactive precursor and accumulates in the bacterium. The use of Bt-toxin as bio-pesticide is not recent. Bt has no adverse effect on animals, birds, aquatic vertebrates and earthworms. Over the years there have been many attempts to market *Bt* as environmentally friendly insecticides but their bio-degradability acts as a disadvantage because Bt must be reapplied at regular intervals during season which increases the farmer's cost. Since then scientists are trying to understand the Cry endotoxin genes from Bt. Current approach is therefore aimed to engineer the crop to synthesize its own toxin. (Schuler, 1998, Crawley, 1993, 2001)

The first attempts to express Cry IA and Cry 3A proteins resulted in very low level of expression in tobacco, tomato and potato plants. That time it was realized that the prokaryotic gene sequence itself would need to be extensively modified in order to obtain high levels of stable expression. Since 1996 maize and cotton are the only Bt crops that are currently commercially grown in the USA, since the Bt potato has now been discontinued. Bt Cry 9C maize in USA & Europe are marketed as Starlink.

MECHANISM OF ACTION OF BT FORMULATION IN BT CROPS

- The ICP-spore complexes are ingested by susceptible insect larvae
- By gut proteases d- endotoxin becomes activated toxin, consisting of three distinct domain
- Toxins bind to receptors in gut epithelium and pore formation takes place.
- Shortly afterwards Bt spores germinate and bacteria proliferate,
- The gut becomes paralysed and larva ceases to feed.

BT COTTON IN CHINA:

One of the most impressive successes in agricultural Biotechnology is China's experience with Bt cotton, which was released to the country farmers in 1997. It quickly became very popular with the area devoted to it expanding from 2000 ha. to 70,000 ha. in 2000. Farmers using Bt varieties needed 80% less insecticides than non-Bt growers. There were considerable health benefits for the farmers. Only 5% of Bt cotton growers reported poisoning, against 22.7% of non-Bt growers. Overall economic benefit of Bt cotton were assessed at US \$ 334 million in 1999. The success of Bt cotton in China paved the way for further expansion of GM crops in this country. China is major producer of Transgenic soyabean, maize and tobacco also, all crops for which GM traits have been developed elsewhere.

COWPEA TRYPSIN INHIBITOR (CPTI):

Certain strains of Cowpea growing in Africa are resistant to attack from a range of insect pests. The isolated insecticidal protein was found to be trypsin inhibitor, very efficient against Lepidoptera, Orthoptera, Coleoptera and was not toxic to mammals. For the initial trial the gene was inserted into Tobacco and transgenic plants produced were tested for their ability to withstand attack from a number of different Lepidopteran pests. It was found that transgenic plants were visibly much more resistant to damage than were the control plants.

GOLDEN RICE:

Golden rice is the result of an effort to develop rice varieties that produce provitamin-A (beta-carotene) as a means of alleviating vitamin A (retinol) deficiencies in the diets of poor and disadvantaged people in developing countries. Because traditional rice varieties do not produce

provitamin-A, transgenic technologies were required.

Provitamin-A is not produced by traditional rice varieties. However, geranylgeranyl diphosphate (GGDP), a compound naturally present in immature rice endosperm, with the help of several enzymes not normally found in rice can be used to produce provitamin-A. Two genes from daffodil and one from the bacterium *Erwinia uredovora* were inserted in the rice genome. These three genes produce the enzymes necessary to convert GGDP to provitamin-A in the rice endosperm. When golden rice is ingested, the human body splits the provitamin-A to make vitamin A. (Ye, 2000)

A number of arguments have been deployed against golden rice. One is that level of provitamin A in the rice is only sufficient to meet about 20 % of the daily requirements for Vitamin A and that the Vitamin A deficiency could be better met by encouraging a better, more varied diet of local vegetables (many of which used to be grown more widely but have been replaced by rice).

GENETICALLY MODIFIED HERBICIDE TOLERANT CROPS (GMHT)

GMHT are crops designed to tolerate herbicides sprayed in field for control of weeds.

GMHT crops can be sown as usual, with minimal or no weed control. Weeds be allowed to develop alongside the crop plants, until a time relatively late in the season, when the weeds began to compete seriously with the crop for light, nutrient or water. At that time a single application of a broad spectrum herbicide would kill off all the weeds, leaving GMHT crop unaffected, so there is less use of hazardous compounds. (Perry, 2001)

TOBACCO PLANTS AS ENZYME FACTORIES

Tobacco was found to be the easiest plant to be genetically engineered. It is a potentially rapid and efficient biomass producer. Crop Tech Corporation, Virginia, USA is a company trying to turn tobacco plants into medicine factories, believe that tobacco plants one day may be able to manufacture human proteins capable of treating different diseases. (Bio Pharming). This company has already inserted nine different human genes into tobacco plants and is testing for desirable proteins. In future tobacco plants may be genetically engineered to make

- Anti cancer agents
- human vaccines
- therapeutic antibodies
- food additives
- human growth hormones

Tobacco is also considered a better choice over transgenic animals like goats and pigs presently used to make human proteins. Because animals have the potential of contamination with viruses. This possibility is very less when the source is a plant. Above all, protein from tobacco plants can be relatively easily separated using relatively simple separation processes than those required for protein of animal source.

BIO-PHARMING

Bio-pharming is the production of pharmaceutical proteins in genetically engineered plants. Proponents of this technology claim that pharmaceuticals can be made in plants at a significantly reduced cost compared to current production methods. Major concerns with bio-pharming are that food or feed crops may become contaminated with pharmaceutical products, and that the products may have negative effects on natural ecosystems.

The manufacture of pharmaceutical products in plants has been among the promised benefits of plant genetic engineering for nearly 20 years "Plant-made pharmaceuticals" (PMPs) are produced by genetically engineering plants to produce specific compounds, generally proteins, which are extracted and purified after harvest. The most common PMP crops that have been grown in U.S. field trials are corn, tobacco, and rice. Other crops being investigated include alfalfa, potato, safflower, soybean, sugarcane, and tomato. Suitable host plants must be easily engineered, be capable of high levels of protein production, and have appropriate procedures for extracting the PMP from plant tissues. Knowledge of the agronomy, physiology, pests and diseases of a crop is also an advantage. Ideally, the host plant would be a non-food crop that does not have wild relatives present in the production environment and could not survive in the environment from seeds carried by wind or wildlife. Another desirable feature is a biological mechanism (such as self-pollination or male sterility) that minimizes pollen drift. Most bio-pharming applications target production and storage of the engineered product in seeds, which naturally accumulate high concentrations of proteins and oils. Seeds are also the easiest part of the plant to store and transport.

RISKS OF GM CROPS

There is a considerable degree of public concern in many parts of the world regarding GM crops.

Critics argue that the newly created GM species could behave in unpredictable and uncontrolled manner. They could even threaten existing species and pollute the environment. (Cherfas, 1991, Straughan & Reiss, 1996, Ryan, 1999, Firbank, 2001) Three major risks are suspected by mass scale cultivation of GM crops:

1. Risk to human health through eating genetically modified food
2. Unforeseen events caused by mobility of transferred gene from new GM crops into other species
3. Deleterious environmental outcome on the arable ecosystem and associated wildlife.

In addition to these Herbicide-resistant gene could be transferred to weeds and create 'super weeds'.

In long run insect larva may develop resistant gene against the toxic protein of Bt.

Bt EFFECT

- By adoption of Bt crops, non-pest insect populations are benefited due to less use of chemical pesticides (a positive effect)
- Build up resistance in pest population
- A report in 1999 states that, pollen from Bt maize was toxic to larvae of Monarch Butterfly, America's most colourful and familiar native (Losey, 1999, Sears, 2000)
- It was found that Bt 176 crops had high level of Bt proteins in the Pollen.
- Bt effect is no longer available in US maize varieties

RISKS OF PLANT MADE PHARMACEUTICALS(PMP):

- The introduced gene or its product may have negative effects on the natural environment. For example, wildlife feeding on the crop may ingest harmful levels of the PMP, or soil micro-organisms may be inhibited by decomposing crop residue or substances exuded from roots of PMP plants.
- Farm workers may be exposed to unhealthy levels of a biopharmaceutical by absorbing products from leaves

through their skin, inhaling pollen, or breathing in dust at harvest.

THE COPY NATURE STRATEGY

Due to the accelerating use of Bt technology to provide insect resistance, rapid appearance of resistant pests was widespread during the first commercial growing season. (it requires a small number of significant mutations in the insect gene).

Effects of Bt crops on non-pest insects (insects which don't damage the crops, are also dying by ingesting the *Bt* protein of crop). Two fold safety issues have also emerged.

Some of the problems encountered with Bt have led some scientists to follow '**Copy nature strategy**'.

- i) Identification of leads : Look in nature for plants that show resistance to insect pests, can be found in the literature, world seed collections or observation in the field. It provides the starting points for the discovery of plant genes that confer resistance to insect damage.
- ii) Protein Purification : Next stage of the process is purification of protein with insecticidal properties. Partial sequencing of the purified protein is one method for isolating the corresponding gene. Characterisation of the protein may also provide means of identifying the gene.
- iii) Artificial diet bioassay : It is important to determine the activity of the isolated protein against the target insect pest by performing feeding assays in the lab. In other words, the isolated protein is effective toxin to kill the target insect
- iv) Mammalian toxicity testing : The toxicity of the protein against mammals (human) should be tested prior to any insertion of the gene into a crop plant. It would be a waste of time inserting a gene into plants that subsequently was found to cause concerns about food safety.
- v) Genetic engineering : After testing its toxicity, its insertion would be considered valuable for transfer to crop plants. Choice of 'promoters' for gene expression is also considered
- vi) Selection and testing : After engineering, selection of transgenic plants, confirmation of transformation,

inheritance of transgens in T_1 , T_2 etc. generation and testing of expression levels needed to be carried out. The effectiveness of the construct (vector carrying the desired gene) then has to be evaluated by insect feeding assays.

- vii) Biosafety : The effects of the transgene on crop yields, insect damage and the wider ecosystem should be properly evaluated in field trials, rather than after several years of commercial planting as occurred with BT crops.

The aim of the 'Copy Nature' strategy has been stated as 'insect pest control which is relatively sustainable and environmentally friendly'. The strategy recognizes a complex interplay in biological communities, between plants, animals, microbes, the soil and the physical environment. It is not just a case of crop plant verses insect pest.

INTERNATIONAL STATUS

- GM food or crops on sale in supermarkets is imported mainly from the US Biotechnological companies like Monsanto, Dupont, Aventis and Novartis
- At present US is the largest producer of GM crops with 20 million hectares, but sends GM crops to the third world.
- Other major growers are Argentina, Canada, Australia, China and Mexico.
- Four major crops grown in these countries are corn, soya, oil seed rape and cotton.

GM CROPS IN UK

- Lack of public acceptance of GM crops in Europe.
- UK made the regulatory process for the release of GM crops into the environment.
- No acceptance with the results of a series of FSEs (Farm Scale Evaluation) were known.
- If no risks to the environment, only then there will be commercial planting of GM Crops.

NATIONAL STATUS

Despite warnings, India bent on GM crops.

- Bt cotton cultivation on 18,000 hectares in Gujrat Agricultural University, run by State Govt..

- IARI, New Delhi developed Bt cabbage the 'Golden acre', resistant against the most dreaded pest, the diamond-back moth.

FUTURE DEVELOPMENT: FUTURE PROSPECTS OF GM CROPS

- Technology for chloroplast transformation be adopted because chloroplasts are normally inherited maternally.
- Gene transfer practice through pollen could be reduced.
- Use of selectable markers could be avoided and transformed plants could be selected by PCR techniques.

As a conclusion, GM crops may have great potential to improve farming and solve major problems of hunger, malnutrition and protection against mutagenic efficacies of Agrochemicals.

Further, In USA, Biotechnology Industry Organization, is currently evaluating three PMPs in clinical trials, which are designed to target non-Hodgkins lymphoma, cystic fibrosis, and E. coli/traveler's diarrhea, respectively. These PMPs are expected to be released for marketing in near future.

UN STATEMENT ON GM FOOD AID

- Populations of countries in Southern Africa are facing a devastating drought leading to starvation.
- UN agencies establish a long-term policy for food aid involving GM food.
- Ultimate decision regarding the acceptance and distribution of GM food rests with concerned Government.
- In spite of the efforts of the UN, there exists confusion over the long term health consequences of GM foods.
- UN can only persuade the hungry, but can not predict the health consequences.
- The argument is why cannot they die of eating GM foods at a later stage instead of dying today of starvation.
- This seems logical, but here ethics are more relevant than logic.
- There are arguments that US want to test the long term health consequences in the name of aid
- In this case recipients will become experimental animals in the open laboratories.

REFERENCES

Beringer, J. (2000). Releasing genetically modified organisms: will any harm outweigh any advantage? *Journal of Applied Ecology*; **37** : 207-214.

Broer, I., (1996). Horizontal gene transfer, stability of DNA and expression of transgenes : in : *Transgenic organisms and biosafety* (eds. Schmidt & Hankeln) Springer-Verlag, Berlin. pp. 67-70.

Cherfas, J. (1991). Transgenic crops get a test in the wild. *Science*, **251** : 878.

Christian Aid (2000). Policy Briefing on Biotechnology and Genetically Modified Organisms, January (2000). Christian Aid, London.

Crawley, M.J., (1993). Ecology of transgenic oilseed rape in natural habitats. *Nature*; **363** : 620-623.

Crawley, M.J. (2001). Transgenic crops in natural habitats. *Nature*; **409** : 682-683

Davies, K., (2001): What makes genetically modified organisms so abhorrent? *Trends in Biotechnology* (in press).

Dewar, A.M., (2000). Delayed control of weeds in glyphosate-tolerant sugar beet and the consequences on aphid infestation and yield. *Pest Management Science*; **56** : 345-350

FAO/WHO Joint Expert Consultation (2000). Safety aspects of genetically modified foods of plant origin. World Health Organization, Geneva.

Firbank, L., (1999). 1st Interim Report on the Effects of the Management of Field Scale Releases of GM Herbicide Tolerant Crops on the Abundance and Diversity of Farmland Wildlife, November 1999.

Firbank, L., (2001). The Farm Scale Evaluations of genetically modified herbicide tolerant crops—an overview. To be submitted to *Journal of Applied Ecology*.

Gebhard, F. & Smalla, K. (1999). Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. *FEMS Microbiology Ecology*, **28** : 261-272.

Hails, R.S., (2000). Genetically modified plants—the debate continues. *Trends in Ecology and Evolution*, **15** : 14-18.

Harpenden, Herts. and Broer, I., (1996). In: *Transgenic organisms and biosafety: horizontal gene transfer, stability of DNA and expression of transgenes* (eds. Schmidt & Hankeln). pp. 67-70 Springer-Verlag, Berlin.

IIASA, (2000). Global Review of Commercialized Transgenic Crops, (1999); ISAAA, Ithaca, NY.

Jones, G. (1999). Genetic Engineering: In *Risk! Life is a Risky Business*, Volume XIII in the "Christ and the Cosmos" Series (ed. P.A. Beetham). pp. 148-170 Springer-Verlag, Berlin ISBN.

Losey, J.E., Rayor, L.S. & Carter, M.E. (1999). Transgenic pollen harms Monarch larvae. *Nature*: **399** : 214

MAFF (1998). Reducing Agrochemical Use on the Arable Farm. MAFF, London.

Mehta, A., Nag, K.K. & Ahmad, J. (1998a). Regeneration of plants of Finger Millet (*Eleusine coracana* Gaertn.) by tissue culture method. *Bionature*; **18(2)** : 95-99

Mehta, A., Ahmad, J. & Nag, K.K. (1998b). Tissue culture studies of Finger Millet (*Eleusine coracana* Gaertn.) *Int. J. Mendel*; **15(3-4)** : 91-94.

OECD (1986). Recombinant DNA Safety Considerations: Safety considerations for industrial, agricultural and environmental applications of organisms derived by recombinant DNA techniques. OECD, Paris.

Oxfam (1998). *Biotechnology in Crops: Issues for the developing world.* Oxfam, Oxford.

Perry, J.N., (2001). Design, analysis and power of the Farm-Scale evaluations of genetically-modified herbicide-tolerant crops. To be submitted to *Journal of Applied Ecology*.

Poppy, G.M. (2000). GM crops: environmental risks and non-target effects. *Trends in Plant Science*, **5** : 4-6.

Ryan, A., (1999). *Genetically Modified Crops: The Ethical and Social Issues.* Nuffield Council on Bioethics, London.

Schuler, T.H., (1998). Insect-resistant transgenic plants. *Trends in Biotechnology*; **16** : 168-175.

Scott, S.E. & Wilkinson, M.J. (1999) : Low probability of chloroplast movement from oilseed rape (*Brassica napus*) into wild *Brassica rapa*. *Nature Biotechnology*, **17** : 390-392.

Sears, M.K., (2000). Preliminary Report on the Ecological Impact of BT Corn Pollen on the Monarch Butterfly in Ontario, March 30, 2000. Canadian Food Inspection Agency, Nepean, Ontario.

Straughan, R. & Reiss, M. (1996). *Ethics, Morality and Crop Biotechnology.* ISBN 0708405703. BBSRC, Swindon.

The Royal Society (1998). *Genetically modified plants for food use.* The Royal Society, London.

The Royal Society and Other National Academies (2000). *Transgenic plants and world agriculture.* The Royal Society, London.

Ye, X. (2000). Engineering provitamin A (b-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, **287** : 303-305.