THREE DECADES OF EXPERIENCE WITH GENETICALLY ENGINEERED CROPS: WAY FORWARD IN THE INDIAN CONTEXT

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Introduction

An important outcome of the elucidation of the double helix structure of DNA is the beginning of the science of genetic engineering. Genetic engineering has made it possible to transfer genes across sexual barriers. However there are several of environmental, biosafety and other problems associated with the technology. Therefore, we must have a very good mechanism for measuring risks and benefits. The report submitted to the Government over ten years ago, one of us (MSS) had pointed out that, "*The bottom line of our national agricultural biotechnology policy should be the economic well being of farm families, food security of the nation, health security of the consumer, biosecurity of agriculture and health, protection of the environment and the security of national and international trade in farm commodities"*.

The traditional plant breeding based on Mendelian laws follows the natural process of sexual reproduction among plants closely related by descent. Variation of genes ('alleles') for recombination of different traits in nature arises from mutations. While mutations to a large extent and alterations in the structure and number of chromosomes of somatic complement to a lesser scale generate variability, the natural selection plays the key role in favouring some of the new mutants to flourish, and rejecting the rest. Plant breeders often encounter '*reproductive isolation'* in distant crosses and this is a barrier to gene exchange even among those plants with morphological similarity. From an evolutionary perspective, onset of reproductive isolation suggests initiation of divergence to form a new species.

The rich biodiversity of the planet Earth would not have come about but for 'reproductive isolation' to gene exchange. The genetic engineering, on the contrary, demolishes the reproductive isolation. The recombinant DNA technology uses stressful processes by which the desired gene (i.e. trait) from any living biological organisms could be 'inserted' into recipient organisms. It differs from Mendelian breeding in atleast two major respects: (i) insertion of genes from widely unrelated organisms into the cells of recipient organisms by means other than sexual reproduction, and (ii) adding a gene (DNA) from outside into the recipient genome, thus increasing the DNA content of the cell from outside. While the term 'Genetic Modification' is appropriate to Mendelian breeding inclusive of mutation breeding, it is indeed incorrect to extend it to genetically-engineered organisms. Yet, the term 'GMO' (i.e. genetically modified organism) is taken to refer to genetically engineered organism as well. This is a clever ploy since the term 'genetic engineering' applied to food crops instils a fear and negative impact in the minds of the public. Further, some biotechnologists or those inspired by modern biotechnology equate the rDNA technology to '*Promethean'* success – similar to Prometheus, mythological giant deity who stole fire from heaven and gave it to humankind. It is known that fire did not have to be stolen from the heaven, as the planets derived from stars which are fireballs fuelled by fusion of hydrogen have innate sources of fire.

Historically speaking, the first genetically engineered crop plant was produced in 1982, an antibiotic-resistant tobacco plant[1]. Marc Van Montagu and Jeff Schell

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established the company **"Plant genetic systems"** (Ghant, Belgium) in 1987. This was the first company to genetically engineer insect-resistant tobacco plants by inserting genes that produced insecticidal proteins (Cry toxin proteins) from *Bacillus thuringiensis*. The first genetically engineered crop approved for sale in the U.S. in 1994 was the 'Flavr Savr' tomato. It had a longer shelf-life, because it took longer to soften after ripening. In the development of Flavr Savr tomato, Calgene Company used recombinant DNA techniques to introduce an antisense polygalacturonase (PG) gene. The PG gene encodes the enzyme PG which is involved in the breakdown of pectin. The antisense PG gene suppresses the production of the PG enzyme and this results in ripe fruit to remain firm for longer period of time. The Flavr Savr tomatoes also contained marker genes that gave resistance to the antibiotic kanamycine which is used in medicine. The Food and Drugs Administration, USA (FDA), ignored many of its own scientists who were concerned that toxicological evaluation of Flavr Savr tomatoes had shown that these had a potential to induce gastro-intestinal lesions/ulcers. Calgene's short term (28-day) studies with feeding Flavr Savr revealed the occurrence of stomach lesions in the experimental rats. Yet, Flavr Savr was cleared for marketing in 1996. Several of the consumers developed gastric lesions. Flavr Savr was withdrawn from market in 1998. The reason given by the developer, however, was not the adverse health effects, but something about marketing problems. In another case, pigs were genetically engineered with human growth hormone so that the pork would have less fat (i.e. lean pork). These pigs called 'Beltsville pigs' suffered from serious bone deformities (i.e they could not stand up and walk) and also severe cardiac problems. So, the production of Beltsville pigs was given up. In the 1990s, yet another case of failure of genetic engineering was that of L-tryptophan. Normally L-tryptophan is produced by fermentation process and has been consistently safe. This is a rather slow process and therefore, it was thought that rDNA technology would accelerate it. So, the gene for L-tryptophan was engineered into *E. Coli* to produce substantially increased quantities in relative shorter time. It was taken for granted that L-tryptophan produced by genetic engineering would be as safe as that by fermentation. However, one batch of genetically-engineered L-tryptophan caused death of 37 people and paralysis of about 1500 people. Litigation amounting to couple of billion dollars is yet to be settled. Notwithstanding several such reports of adverse health effects, the insecticide (BT) and herbicide (HT) transgenic crops were vigorously promoted in a few countries (USA, Canada, Argentina, Brazil, India and China) whereas European Union, Japan and most others have taken a cautious approach using the precautionary principle.

Inadequate Understanding of Molecular and Cellular Events Following Insertion

The central dogma of molecular biology namely DNA $\rightarrow$ mRNA $\rightarrow$ Protein has undergone considerable refinement and better understanding over the decades since it was proposed in late 1960s. Cellular phenomena such as post-translational modification, epigenesis in gene expression etc., are known to play determinant roles in the synthesis proteins. To begin with, the processes of cell culture, isolation of the desired gene (DNA) from any living organism and inserting it into the recipient cell by mechanical and/or biological devices are all stressful. No one knows exactly the impact of these stressful operations on the native genome of the cells *in vitro*. Then comes insertion, which does not happen at a specific site in the genome. The insertion occurs at random. The genes adjacent to the site where the trans/cis gene has been inserted undergo instability that often reflects as 'insertional mutagenesis'. There have been numerous reports of "unintended effects" in the genetically engineered crops. A very early report of 'unintended effect' was made in 2005[2]. This paper demonstrated that an enzyme alpha-amylase inhibitor-1 from common bean (*Phaseolus vulgaris*) becomes an allergen when the gene for alpha-amylase inhibitor is engineered into pea (*Pisum sativum*) genome. It was found that in pea, a structurally modified form of this inhibitor was formed. The authors concluded that transgenic expression of non-native proteins in plants may lead to synthesis of structural variants possessing altered immunogenicity. A. K. Wilson *et al.* have reviewed the transformation – induced mutations in transgenic plants.[3] The report of unintended compositional changes in transgenic rice seeds[4] causes deep concern. For instance, the 'Golden rice' might provide enhanced pro-vitamin A, but the question is about its altered composition. This question has not yet been addressed. Logically, rice is not a source for vitamin A. Rice is eaten for its carbohydrates and fibre; and additionally, in some varieties, medicinal properties are of value. What is needed is to enhance awareness of the rice-dependent population to include green leafy vegetables, carrots, beet root etc., in their diet to combat vitamin A deficiency. There is, however, no doubt the development of Golden rice is remarkable feat in modern biotechnology, but its social objectives need thorough reexamination.

What We Need, but What We Have Got

Apart from problems arising from incomplete and inadequate understanding of the basic molecular and cellular processes involved in genetic engineering, a major health and environmental crisis has also emerged with production and release of transgenic crops which produce Bt-cry proteins (Bt-crops) to shield against Lepidopteron borer pests (e.g. cotton boll worm (*Helicoverpa armigera*), pink bollworm (Pectinophora gossypiella), fruit and shoot borer of brinjal (*Leucinodes orbonalis*) etc.,) and herbicide-tolerant (HT) transgenic crops. The most basic problem with the Bt-transgenic crops such Bt-cotton, Bt-soybean, Bt-corn etc., is that lower concentrations of the cry proteins in the plant tissues do not kill the larvae; instead the low concentrations induce 'selection pressure' which in turn leads to induction of mutations in the pests to develop resistance (i.e. genetic shield) against the toxic Cry proteins. Once these pests become resistant to Bt-toxin, they attack the Bt-transgenic crops as they do the non Bt-counterparts. When the Bt-transgenic cotton (Bollgard I) failed, the Multinational Company developed Bollgard II with additional category of Bt Cry proteins. A couple of years ago, Bollgard II also came under severe attack by pink boll worm which had developed resistance. Consequently, cotton farmers cultivating Bollgard II experienced huge losses. And the resource-poor cotton farmers having lost their crop and livelihood resorted to committing suicide. A. P. Gutierrez *et al.* have shown that annual suicide rates in rainfed areas is directly related to increases in Bt cotton adoption (i.e. costs)[5]. So, when the Bt-cotton becomes susceptible to the borer pests, the farmers have got to spray chemical pesticides. This tremendously enhances the cost of inputs. Since in India the Bt-trait has been introduced into American (tetraploid, 2n=4x=52) hybrid cotton, the resource-poor farmers are not able to save seeds for sowing in the subsequent season, and have got to buy seeds for sowing afresh every year at the cost fixed by the company. Further, the inputs such as chemical fertilizers, copious irrigation, weeding etc., greatly enhance the cost of cultivation. And often, they do not get market price commensurate with enhanced cost of cultivation.

There is a basic question as to what made India to adopt the Bt- transgenic cotton technology from the USA. Among several reasons against the introduction of Bt-transgenic hybrid cotton, one is that maintaining a 'refuge zone' around the GE crop field to limit resistance development is not feasible for small and marginal farmers. The 'refuge' zone involves sowing several rows of non GM cotton around the transgenic Bt hybrid crop. In the USA, the farm sizes are huge with several hundred acres or so, and sacrificing a few acres for raising 'refuge' would not economically hurt the farmers. On the contrary, a large proportion of cotton farmers in India are resource-poor small and marginal farm holders. They cannot afford to sacrifice even a couple of rows from their holding of about 1 hectare or even less to raise 'refuge' crop. The blame put on these farmers for not raising refuge in their Bt – hybrid cotton fields is quite unfair. Their economic distress got multiplied also because of infestation by pests other than borers as well. Whitefly attack on Bt-cotton became serious in some parts. The consequence of all these is to increase the amount of chemical pesticides used. Dr. Keshav Kranthi, former Director of Central Institute for Cotton Research (CICR) Nagpur has written an article "Fertilizers gave high yields, Bt only provided cover" (*Cotton Statistics and Views* 2016 – 2017, No. 39, 27 December 2016). He brings out that as the area under Bt-hybrid cotton cultivation increased from about 6.7% in 2004 – 2005, to about 97% in 2011, the yield reduced from about 550 to 610 kg/ha to about 486 kg/ha. The use of insecticide which was quite reduced in 2003 – 2004 has now risen almost to the level of pre-Bt era. He concludes "*Bt cotton was supposed to have conferred two major benefits to cotton production. (i) High yields due to effective protection of bolls from bollworm damage and (ii) Reduction in insecticides recommended for bollworm control*". Official data show that none of these two promises were kept in the past 10 years in India. It is dismal that cotton production progress in India has hit a dead-end over the past 10 years.

The experience with herbicide-tolerance transgenic crops (HT – soy; HT – corn) in the USA is even worse. The most commonly inserted gene to make transgenic corn, soy and cotton etc., is the herbicide (glyphosate-based Roundup) - tolerance gene. As early as 2004, one of us (MSS) in his capacity as the Chairman of the Task Force in Agricultural biotechnology had cautioned against engineering the HT- trait (i.e. herbicide tolerance) that would cause not only environment and health hazards but also substantial reduction in rural livelihoods (by taking away the opportunity to earn wages by manual weeding) especially by landless rural women[6]. That glyphosate is a potent teratogen (i.e. an agent that causes malformations in the developing embryos) has been shown by several studies[7]. The International Agency for Research on Cancer (IARC) in Lyon, France (the cancer-research arm of the World Health Organization) has rated glyphosate as "probable carcinogen to humans, labelled category 2A".

Writing under the title "A Hard Look at GM Crops" asserts that HT-transgenic crops have bred 'superweeds'[8]. Since late 1990s, herbicide-tolerant cotton was a success until about next ten years. In 2004, herbicide-resistant amaranth (a weed) appeared in one county in Georgia and by 2011, it had spread to 76 counties. As of 2012 twenty-four glyphosate-resistant weed species have been identified across the USA. Glyphosate-resistant weeds have now been found in 18 countries worldwide, with significant impacts in Brazil, Australia, Argentina, and Paraguay. The advice from Monsanto, the company that manufactures Roundup and has also developed HT crops is that the farmers should start ploughing and using a mixture of herbicides. In the context mentioned above, India should take an appropriate policy to ban all the HT crops. However, on the contrary, the Genetic Engineering Appraisal Committee, (GEAC), MoEF Government of India has approved the herbicide-tolerant (glufosinate) hybrid mustard DMH-11. The Technical Expert Committee (TEC) appointed by the Honourable Supreme Court of India in its report submitted in 2013, had recommended total ban on HT-transgenic crops. The reasons were primarily on grounds of its adverse health effects, its exertion of 'selection pressure' to form 'superweeds' among species constituting the rich biodiversity. The HT transgenic mustard, if commercialized, could lead to genetic pollution of an extremely rich biodiversity of the genus *Brassic*a, and the havoc that Brassica 'superweed' species would cause in thousands and thousands of resource-poor small and marginal farms would be far beyond control and restoration. Indian agriculture is quite different from that in USA.

Corporate Call for Community Participation

Unethical though, the developers of transgenic BT and HT crops ultimately choose the illiterate and resource-poor farmers either to put the blame on, or to try to find solutions for the faulty technology created by them. There are atleast two instances both from India. The first is about the HT transgenic hybrid mustard DMH-11. The use of herbicides, such as glufosinate is banned in India. Since the DMH-11 requires the use of glufosinate to selectively kill the male-fertile lines (which do not possess the HT gene), it is an integral part of the procedure. It also occurred to the GEAC that some enthusiastic farmers might spray glufosinate on the DMH-11 in the field to kill the weeds and that would create legal and social problems. A recent report in '*The Hindu'* (Thursday, July 27, 2017) carried a news item with the title, "Panel for Action Against Farmers Using Herbicides on GM Mustard"! The question is as to why the scientists should in the first place develop a herbicide-tolerant crop, when the application of glufosinate herbicide is not legal. After developing such a technology, it is totally unfair to put the blame on the poor farmers.

In yet another instance, following the resistance to Bt toxin developed by pink boll worm in Bollgard II and the imminent collapse of Bollgard II cotton, a commentary by K. S. Mohan is noteworthy.[9] In this Commentary title, "An area-wide approach to pink bollworm management on Bt-cotton in India: a dire necessity with community participation", the author would want the Bt-hybrid cotton farmers to adopt 'integrated pest management (IPM)' in order to manage the problem caused by the failure of Bollgard II consequent upon development of resistance to Bt-toxin by the pink bollworm. This Commentary confirms that the expensive Bt-transgenic technology has failed within about 6-8 years of its introduction and thus it is not a sustainable solution to the management of pests. Secondly, an expensive and failed technology would now want to ride on the back of a time-honoured, substantially inexpensive traditional technology.

Do We Need Genetic Engineering Technology at all in the Realm of Plant Breeding?

As has been emphasized by one of us (MSS), in 99% of the situations, Mendelian breeding that is less expensive and also in tune with nature would be effective to achieve the goals. The importance in this context is the conservation of biodiversity. There is availability in nature of genes to shield the cultivated crops again not only 'biotic', but also 'abiotic' stresses. However, the genetic engineering in plant breeding could be resorted to where it is not absolutely possible to shield crop plants against extreme abiotic stresses resulting from climate change. For many crop species, the wild relatives do have genes for abiotic stresses such as salinity, drought, submergence etc.

In fact, the form of agriculture needed to sustain food and nutrition security for the present and future has to be both eco-friendly as well as pro-women, pro-poor, and pro-livelihood oriented. Today, there is substantial evidence that 'organic' agriculture and its modified version of 'green agriculture' could provide consistently high yields over long periods of time. The concept of 'evergreen revolution' proposed and developed by MSS defines it as "*achieving productivity in perpetuity without accompanying ecological and social harm"*. This definition precludes the use of BT and HT transgenic crops because they break down due to development of resistance by pests and are not therefore sustainable. Even more importantly, most pesticides are endocrine disruptors, teratogens, genotoxins, and carcinogens. The adverse impact of chronic exposures, if any, could not be assessed in short term (i.e. 14 days or 90 days) studies as genetic effects such as malformations of fetus, cancer etc., take a long time to manifest. These short term tests serve little purpose. Most pesticides (including BT and HT) are endocrine disruptors like DDT. Recently, it was shown that the carcinogenic effects of DDT manifest only after about five decades. Many short term studies during the 1950s and until its ban in 1962 had revealed DDT as a clastogen, but not as a human carcinogen. The elegant studies by B. A. Cohn *et al* showed that dichloro diphenyl trichloroethane (DDT), an endocrine-disruptor induces breast cancer in women of about 52 years of age, following their exposure *in utero* in the 1960s[10]. Their mothers who had high exposures to DDT in the 1960s showed high levels of DDT in their system. It is an amazing study of 54-year follow-up of mothers exposed to DDT in the 1960s, the consequent exposure of the female foetuses in such pregnant mothers, and these daughters exposed to high levels of DDT in their mothers' wombs developing breast cancers when they reach about 52 years of age. The study showed that women exposed to the higher levels of DDT in the womb had 3.7 times higher risk of breast cancer than those who had the lowest exposure to DDT. Therefore, it is highly inaccurate to conclude that short-term studies did not reveal adverse effects. What is needed is long term studies in rats for atleast two years – the minimum time required to assess the tumour-induction. In food crops, the studies should be extended to the next couple of generations as well. In principle, transgenic crops which directly or indirectly influence the use of pesticides should NOT be developed at all.

The other concern is that crops and traits have so far been chosen by developer and is therefore 'top-down' in nature. In doing so, the commercial interest of the developer(s) outweighs the farmers' needs and ecological concerns. So, the first step in the decision making process should be to short list crops and traits for genetic engineering and whether the objective(s) could not be achieved through Mendelian breeding or mutation breeding etc., which do not involve addition of gene(s) into the native genome of crops. The basic need is the conservation of genes for future genetic shielding of agri-horticultural crops in the human-made epoch Anthropocene.

Biodiversity - Feedstock of Biotechnology

India needs more importantly a Department of Biodiversity Conservation than a Department of Biotechnology. The importance of biodiversity conservation has been explained in different ways by MSS for over six decades. MSS was the President of the XV International Congress of Genetics held in New Delhi in 1983. His Presidential Address was "Genetic Conservation: Microbes to Man"[11]. Among other issues, he made a plea for international efforts in cryo-preservation of seeds and propagules for future needs. His plea was realized in the form of Svalbard Global Seed Vault set by Norwegian Ministry of Agriculture, but managed jointly by the Norwegian Ministry of Agriculture, The Global Crop Diversity Trust, and Nordic Genetic Resource Centre. Located in the village of Longyearbyen on Svalbard Island situated at 78^0C north Arctic Circle, vaults were chiselled out in icy mountain to store sample seeds and vegetative propagules of about 4.5 million plant species and varieties. The vaults have a natural temperature of -4^0C round the year, which is further lowered to -1.8^0C, the optimal temperature for long-term seed viability. In February 2009, the Norwegian Government organised a seminar on "Frozen Seeds in a Frozen Mountain". MSS gave an invited lecture, "Freezing Seeds: A Humanitarian Issue". Almost in parallel, the Defence Research and Development Organisation (DRDO) of India have established a similar seed storage facility under permafrost conditions at Chang La in Ladakh.

Genetic engineering has created a false impression on the minds of young students in the past 2-3 decades that modern biotechnology can solve all the present and future problems. Unfortunately, it also led to increasing neglect of the biodiversity conservation. Aware of all these, MSS emphasized in several of his writings and lectures that 'Biodiversity is the Feedstock of Biotechnology'.

Yet, another issue of deep concern regarding genetically engineered crops is the health and environmental safety. India does not have an authentic and independent food safety evaluation system for genetically engineered crops. What happens at this time is that the developers of transgenic crops are also the ones to provide biosafety data which are simply accepted by the GEAC. The Department of Biotechnology Government of India should lead and support food safety evaluation of all foods especially the genetically – engineered crops. This aspect had been dealt at length in the Report of the 'Task Force on Agricultural Biotechnology' chaired by MSS.

It has been scientifically proven that the notion of 'substantial equivalence' has no scientific evidence at all, and it was introduced by the developers of GE crops as a convenient excuse to escape from the truth about the adverse health effects especially of pesticidal transgenic crops. Instead of erring on the safer side which is prudent in the event of any uncertainty, **"Precautionary Principle"** should be strictly adhered to. The notion of substantial equivalence should be outright rejected.

Farming System for Food and Nutrition Security in Epoch Anthropocene

The Nobel Laureate Paul Crutzen (2002, *Nature*) coined the term *Anthropocene* to the present, in many ways human-dominated geological epoch, supplementing the 'holocene', the warming period of about 10000 to 11000 years. Anthropocene by no means is limited only to climate change; it encompasses drastic changes in the entire Earth system involving nitrogen, hydrologic, carbon cycles etc. What however, is the greatest concern is that anthropocene threatens to tip the complex Earth system out of cyclic glacial-interglacial pattern during which *Homo sapiens* have evolved.

The link of genetically-engineered crops with anthropocene is that henceforth chemical inputs (both inorganic chemical fertilizers and organic pesticides) would have to be drastically reduced. One reason is that the Haber-Weiss process of making ammonia has generated huge amounts of nitrates on land and aquifers without an equivalent chemical method to reconvert nitrates into atmospheric nitrogen. The Earth has today a completely vitiated nitrogen cycle. Before the advent of Haber-Weiss process, Earth had a perfect nitrogen cycle with nitrogen-fixing bacteria fixing atmospheric nitrogen as nitrates and the denitrifying microorganisms breaking the nitrates into nitrogen.

Intensification of agriculture with chemical inputs would only accelerate planet towards a 'tipping point'. The relevance of this statement to modern biotechnology is that several multinational companies which produce chemical pesticides and herbicides are also the major players in developing genetically-engineered BT and HT crops.

The 'Zero Hunger' programme of the United Nations launched at the UN Conference on Environment and Development in Rio de Janeiro in June 2012 aims at total elimination of hunger from the globe by 2025. To this laudable goal, MSS has added '*nutrition security*' as well. More than 600 million people in India alone suffer from 'hidden hunger' caused by inadequate intake of micronutrients and vitamins. World over about two billion people are suffering from nutritional inadequacies. The nutrition security to eradicate "hidden hunger" cannot be achieved through corporate farming system, monoculture and chemical intensification. Instead, the farming system for nutrition (FSN) proposed by MSS ideally integrates the pro-nature, pro-poor, pro-women ad pro-rural livelihood dimensions of (a) farming with landscape, (b) providing livelihoods to the rural landless women (c) cultivating such agro-horticultural plants naturally rich (biofortified) in specific micronutrients/vitamins capable of providing remedies to nutritional deficiencies in a given region. Already, MSSRF has set up gardens with agro-horticultural plants identified for their high content of iron, iodine, zinc, vitamin A, vitamin C etc.

In the design of farming system for nutrition, the genetically engineered crops should be avoided as these are now known to produce 'unintended effects' and moreover, these are neither pro-nature, nor pro-poor, nor pro-women. Therefore, genetically-engineered crops in the Indian situation would be limited to the rarest of rare situations.

An important requirement for FSN is access to genetic material having the desired genes for nutritional characteristics. For this purpose, we should establish a network of genetic gardens of biofortified plants. In addition, it is also important to train local communities in different aspects of nutrition security. Our goal must be to move away from food security to nutrition security. In this area, it is important to ensure that food safety is safeguarded. As already emphasised genetically modified crops would need to be carefully studied from the point of view of nutritional impact and biosafety. ❒

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