Biotic and Abiotic Stressors In Plant Molecular Breeding vis-à-vis Sustainable Molecular Farming Strategies For Food Security: Threats, Opportunities and Challenges Ahead in Indian Perspectives.

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ABSTRACT

We are currently in the midst of the "GOLDEN AGE" of plant biology rather to say "PLANT SCIENCES". Our sincere thanks be extended to the stimulus and support provided by the widespread inventions and applications of empowered techniques from all other interdisciplinary branches of science (Physics, Chemistry, Mathematics, Statistics etc) to plant scientists. We the plant biologists can now boast of claiming us as "the ablest" to elucidate and solve the hardcore challenges of 'crop science'.

In today's world every nation has been revamping their present modus operandi to save the biodiversity resources. Biodiversity caters the feedstock for the biotechnology industries in all countries. Though the developed countries are rich in formulating the research methodologies automatically seek the resources, to supplement their demands, from the third world countries. Obviously serious debates and confrontations are getting mightier which only can and must be resolved amicably irrespective of all sorts of compulsions. This paper aims to explore the key issues raising a current debate for the protection of plant varieties, Genetically Modified Organisms (GMOs), unethical access of biological resources by various multinational companies without prior informed consent (PIC) and no benefit sharing with the concerned communities along with mindless exploitation of wild flora and fauna have become a serious threat for our rich agro-biodiversity, agricultural systems, ecology, soil fertility etc. Positive steps should be taken to arrest the present disturbing trend assuring food security for all.

KEY WORDS: *Food security, GM crops, intellectual property rights, participatory approaches, plant biotechnology, Bt-cotton, terminator technology.*

1. Introduction:

Plant Science, with popular appellation as "Botany" had had its inception during 372-286 B.C. with the altruistic scientific experiments of Theophrastus with the patronage of the-then Roman Kings depicting all the observations in Latin scripture. Botany as the choice of the royal students of the universities in Greece, Rome and Italy could start its proper journey with the advent of microscopes opening up a plethora of disciplines for the students from the last century. With passage of centuries this particular branch of science has metamorphosed voluminously with its pulsating presence with all other branches of modern science to cater the *prima facie* demands for food, shelter and apparel to human civilization.
We all know the "Green Revolution" led by Prof. Norman Bourlaug, Monkombu Swaminathan and Gurdev Khush, enabled the world's food supply to shoot up during the last three decades of the 20th century. In fact in the developed countries food supply increased much faster than the demands. Their technological progress contributed to a decrease in the cost of production so that the farmers were able to share the benefits of the yield with the consumers by offering the food to them at a lower price although forced in a compromised compensation of maintenance of natural biodiversity [1, 5].
But the discovery of the three dimensional structure of DNA by Watson, Creek and Wilkins in 1955, possibly was the best of all the achievements in the biological world during the last century. It portrayed all the possibilities for an understanding in its finest details of the molecular configuration of genes which depicts the general as well as the individual characteristics of all living organisms including plants. The dazzling diversity of the achievements of DNA science ranging from plant genomics to DNA fingerprinting, crop science to frontiers of environmental remediation and this branch of Science undoubtedly has propounded a resounding impact on the humane face of civilization and its progress [3].
The enthusiasm with which the plant biologists have adopted the techniques is encouraging and the way newer branches are ramifying from this mother subject is really astounding. For example the moribund branches like plant morphology and anatomy have been reinvigorated by the growth of interest in "Plant Developmental Biology" and many arduous, dedicated students are once more becoming interested in identifying the key issues in plant morphogenesis. The widespread adoption of *Arabidopsis thaliana* as a model plant and the accompanying growth of interest in transgenic methods have also accelerated the progress resolving many long-standing queries in plant system biology. Genes encoding the proteins that regulate many aspects of growth and development have now been almost known and been characterized by exploiting the genetic make up of *Arabidopsis*. At least 2000 scientists from far-flung corners of the globe are now using *Arabidopsis* as a primary experimental organism and more than 9000 *Arabidopsis* genes have already been sequenced.
But a major challenge faced amidst these great discoveries by the plant biologists is the destructive adaptations of pathogens over their genetically modified host plants. Much work is underway to highlight the molecular basis of the mechanisms by which plants sense the infection by fungal and bacterial pathogens and to register a defensive retort. Scientists are still in the murky lanes unable to detect the molecular basis for the high degree of specificity in the host-pathogen interaction. A tentative hypothesis could be that the plant resistance genes encode membrane receptors which intercept the signals from the pathogens and trigger the cascade of cellular defense responses. Attempts are on track to identify the host factors with their finer nuances that mediate the specificity of interaction between the obligate and parasitic pathogens eliciting pathophysiological manifestations in plants.
Besides being susceptible to destructive infections by an array of viral, bacterial, fungal and nematode species, plants also can participate in a number of benign and beneficial interactive networks. Today it is an established fact that plants have evolved a set of sensing mechanisms that permit/allow them to recognize and respond to pathogens. These mechanisms are quite environment-specific and host-selective where a large number of disease resistance genes confer the resistance upon one species or in some cases to one race of a pathogen.
Today the subject of Plant Science provides an international framework for cooperation among the scientists, researchers, and agriculturists/policy makers in the development of molecular breeding strategies. Crop physiologists and plant biotechnologists from all over the globe with all their research

impetus can now provide a perfect milieu for training the future hands who would respond to the challenges and opportunities ahead [4]. Recently to develop a GM crop two traits (herbicide and pesticide resistance) are a must-to-incorporate in commercial crops to examine the reasons for their success. Plant Science students and researchers lately have avenues for potential exploration on developments in other superlative agrarian traits such as disease resistance, a-biotic stress, metal toxicity resistance, improvements of yield and quality, at their immediate disposal to be nurtured with utmost sophistication. So plants are now explored as the factories of molecular farming for a large scale boosted-up production of neutraceuticals, cosmeceuticals, antibiotics, edible vaccines and antibodies [3a].

2. Plant molecular farming vis-à-vis conventional breeding system:

Fredrick Banting had initiated the stepping stone in the new pavement called conventional molecular farming in 1920 with the extraction of insulin from animal tissues by, as described by [6]. But it had developed several drawbacks in its approach which most likely led to the establishment new molecular farming methods through considering novel sources. New methods were developed accordingly as the availabel molecular sources (e.g., plant cells, transgenic plant cells, virus-infected plants, animal cells, transgenic animals as one of the outcomes of this process, and the major source in this field is the transgenic plant cells [7]. In comparison to the other transgenic products obtained from transgenic bacteria, fungi, and animals, which are the most common models for recombinant-protein production, plant-based therapeutics are produced with the lowest cost, leading to an economic justification from the commercial point of view from producers and also for their affordability for common mass [8]. The benefits of recombinant-protein production using molecular farming using higher plants are manifold. Technically, the stability of recombinant proteins within plants stressed by environmental factors is greater than that of recombinant proteins produced in other functional hosts. Furthermore, higher plants typically produce recombinant proteins with the correct folding, activity and glycosylation [9, 10]. Another beneficial characteristic of these systems is that recombinant plants can be stored at room temperature and easy to maintain in contrast to the storage temperature for viruses, bacteria and yeasts is -20 ºC. The storage condition for cultured mammalian cells is even more stringent because they must be maintained in liquid nitrogen [11]. Among plant species, plants with watery tissues, such as tomato plants, are more suitable for molecular farming than are dry-tissue plants, such as cereals. This phenomenon could be related to the ease of extraction of squashy tissues [12]. Despite all of the advantages of using higher plants, there are limits to the products than can be obtained using plant molecular farming, such as the unknown mechanisms that cause certain post-translational disorders in plant cells. The problematic issues of how to fine-tune the systems that are essential for the preservation of the structural integrity of the nascent recombinant proteins and their activities in their new cellular environments are still debated and challenged within the field of plant molecular farming [13].

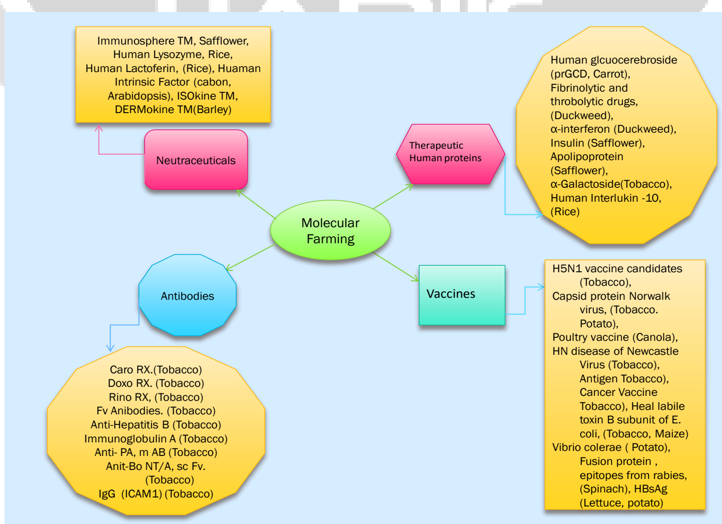

Fig 1: Examples of plant molecular farming products in different types of plants

2.1. Environmental stressors on transgenic plants:

The effects of different environmental factors on the health, biomass production and activity of plants are matters of concern for plant biologists even under normal conditions; however, these factors become more serious concerns during the post-transformational regulatory periods. Indeed, genetically transformed plants are extremely susceptible to the effects of environmental factors immediately after being transferring to natural soil. Environmental conditions have the capacity to directly affect the quality and quantity of recombinant proteins thus produced inside plant cells. The factors that may negatively affect transgenic plants can be categorised into biotic and abiotic stresses. In this regards, light, drought, salinity, nutritional deficiency and cold have negative effects on plant secondary metabolite production. These factors are abiotic stresses [14]. Light plays a crucial role in regulating photosynthesis and the chronological events that drive the growth and developments of plants toward the flowering stage [15]. Generally, plants use light of 400- and 740-nm wavelengths to conduct photosynthesis. Light of these wavelengths is called photosynthetically active radiation. Photons of wavelengths lower and higher than this range are either impractical or destructive for photosynthesis due to their insufficient or extremely high energy levels [16]. To protect recombinant proteins against stresses, scientists are trying to optimize the light conditions, such as the length of the day and the light intensity. Other stresses, such as drought, restrict the overall development of crops. This stress decreases the productivity and quality of plants and leads to morphological changes. Consequently, the expression of recombinant proteins may be down regulated simultaneously. Similar to the effects of other stresses, drought leads to the accumulation of reactive oxygen species (ROS) in plants, causing oxidative stress [17]. Moreover, several physiological traits, such as the carbon-assimilation and stomatal-conductance rates are prominent determinant rate limiting factors of fitness under drought conditions due to their relevance to the efficiency of water-use and photosynthesis [18]. Another restricting factor of plant molecular farming is salinity. Approximately one-third of the world's irrigated farms are ineffective due to the excess salt content of the soil [19]. The adverse effects of salt on plants are manifested in two ways. Initially, a high concentration of salt in the soil directly hampers water absorption by the roots by affecting root-soil osmotic regulation. Secondly, salt accumulation in various organs poisons plants [20]. The two toxic ions derived from NaCl, Na^+ and Cl^-, can damage plant cells through both osmotic and ionic dysbalancing mechanisms [21]. Quantitative and qualitative changes in metabolite synthesis, as well as the occurrence of enhanced metabolic toxicity during stress periods are a few of the most usual physiological impediments of stressed plants [22]. Furthermore, salt stress alters the expression of cell-cycle progression genes through affecting mitotic cell division [23]. All of these cellular metabolic deregulation may be affected by altered hormonal homeostasis occurring under salt-stress conditions [24]. It has been well documented that the abscisic (ABA) content of plants increases under heavy salt-stress conditions [25]. Plants try to adapt to saline stresses by accompanying alterations in the level of numerous metabolites, proteins, and mRNAs [26]. A variety of genes, the expression of which is activated in response to salt stress, after being identified have been transferred to plants [27]. High salinity conditions promote plant-cell dehydration [28] many of the genes that are activated by saline stress are also activated by drought whose expression is regulated by abscisic acid (ABA), a plant hormone that is generated in response to saline stress [29]. Temperature has been another important environmental factor that affects plant growth and productivity. High temperature stress induces certain physiological, biochemical, and genetic changes in plants, making proteins denatured, lipid peroxidation, and perturbation of membrane integrity [30]. High temperatures decrease the rate of synthesis of normal cellular proteins and induce the synthesis of heat-shock proteins [31]. High temperatures are harmful to plant cells, leading to a loss of viability. In *Medicago sativa* transgenic plants, heat shocks can inactivate the transcription of genes encoding phosphinothricin/N-acetyltransferase recombinant proteins [32]. Sometimes, transferring plants to an environment with a stressful heat level initiates the expression of members of heat-shock protein/chaperone cascades that prevent the misfolding, denaturation and aberrant aggregation of cellular proteins/enzymes [33]. Plants lacking the ability for temperature adaptation are supposed to be incapable of inducing structural or functional changes in their proteins. It has been reported that exposure to 25 °C and high light conditions can increase the biomass and total soluble protein content of plants, whereas exposure to high light conditions and 15 °C favoured the production of a recombinant monoclonal antibody by transgenic tobacco plants [34]. The temperature also affects the glycosylation of recombinant proteins (antibodies) in plants. Sulphur (S) is a major component of any protein molecule; hence, its uptake and assimilation can affect the production of recombinant proteins in transgenic plants. Of course, S uptake is dependent upon a constant supply of the precursor of cysteine, O-acetylserine, which in turn, is dependent on the presence of adequate nitrogen and carbon sources [35]. As for example, the combined application of S and N affects the accumulation of lipids in rapeseed (*Brassica napus* L.) via the induced increase in the rate of protein synthesis [36]. The significantly positive correlation of the antibody and total protein contents of transgenic plants allows the prediction of the fluctuating trend of antibody accumulation through monitoring changes in the amounts of total protein. The above-mentioned facts suggest that providing balanced nutrition and proper management of physical factors would enhance the production of pharmaceutical proteins by transgenic plants.

2.2. Heterologous gene transfections in climate-risk-free production systems and biosafety considerations:

Plant biotechnology typically relies on two strategies for delivery and expression of heterologous genes in plants, including a) stable genetic transformation, and b) transient expression using viral vectors [37]. In recent years, the technological progression in virus-based vectors has allowed plants to become a feasible platform for recombinant proteins (RPs) production, while RPs were only able to be produced from cultures of mammalian, insect, and bacteria cells, previously. The plant-based recombinant proteins are now more preferable in terms of versatility, speed, cost, scalability, and safety over the current production paradigms [38]. In spite of being a faster method, the transient approach is hampered by low intake of viral vectors carrying average- or large sized genes inside plant cells. Fortunately, these drawbacks have been subjected to troubleshooting by developing constructs for the efficient delivery of RNA viral vectors as DNA precursors. The mentioned efforts have tended to expanding systemic *Agrobacterium tumefaciens*-mediated transfection of viral replicons for efficient transient expression in plants. The target is to transfect all developed leaves of a plant simultaneously; using *Agrobacterium*-mediated delivery of the target constructs by gene amplification inside the host system. This process is also referred to as "magnifection" that can be performed on a large scale and with different plant species. The mentioned technique incorporates advantages of three biological systems consisting of: a) the transfection efficiency of *A. tumefaciens*, b) the high expression yield obtained with viral vectors, and c) the post-translational capabilities of a plant. This procedure does not entail genetic modification of plants and is faster than other current methods [37]. Transient expression systems have been established to eliminate the long-time frame of generating transgenic plants, so that the transgene is not integrated into the plant genome but rather quickly directs the production of the RP while residing transiently within the plant cell. In addition to the significant acceleration of production timeline, this approach improves the recombinant proteins accumulation level by excluding the “position effect” of variable expression instigated by the random integration of transgene within the genome [39]. In another word, the climate risk free molecular farming systems have become more achievable by conducting the transient gene transfection. Beside all these advances achieved by the transient expression technology, some complementary strategies have been taken into consideration to limit the potential environmental and human health impacts linked to PMF (Plant Molecular Farming). Specifically, cell cultures of transgenic plants, physical containment, dedicated land, plastid transformation, biological confinement, male sterility, gene use restriction technologies (GURTs), expression from or in roots, expression in edible parts and seeds, post-harvest inducible expression, and temporal confinement have been suggested as additional solutions to minimize the risks of Plant Molecular Farming [40].

2.3. Plant Molecular Farming: current status and perspectives

As plant molecular farming has flourished in leaps and bounds, there have been technological progresses on many aspects, including transformation methods, regulating gene expression, protein targeting and accumulation, as well as the use of different crops as production platforms [41]. Recently, plant molecular farming has been proposed as an example of a green development scheme in convergence with sustainable agricultural industries. The advantages of transgenic plants over other expression systems make them become industrialized as economic alternatives to the conventional pharmaceutics. Several plant-made pharmaceuticals, including the enzyme glucocerebrosidase (GCase), insulin and Interferon alfa 2b [IFN-alpha (2b)], have approached commercialization with low costs and large-scale production. Interestingly, these achievements have been attached to substantial patenting activities as well. Reportedly, there was a tangible downward trend in the number of patents filed from 2002 to 2008, and a greater number of patents were filed by public sector institutions or inventors than by the private sector [42]. The USA dominated patenting activity providing nearly 30% of inventors. Most of the patents were related to vaccine candidates (55%), followed by therapeutics and antibodies with 38 and 7%, respectively [42].

3. Indian Scenario: some case studies

Recently, massive crop losses have been reported in Punjab, India, by the white-fly to the Bt-cotton crop. Most of the marginal farmers had grown Bt cotton and large scale damage to cotton crops has also been reported from parts of Haryana and Rajasthan, India, also recently. Genetically modified crops like Bt cotton were supposed to offer protection from pests. Contrastingly, the protection of commercial crops remained unanswered in India in recent times as crops over huge are destroyed by ball worm or white fly or from attack of other pests. The farmers were informed well in advance that GM crops comes with built-in protection from a wide range of pests and the cost of expensive pesticides would get reduced. But in reality peasants had to spray more and more for the pests leaving them cheated. This is not just the experience of a few regions of India. After probing and amassing evidences all across the world on GM crops a vociferous panel declaration was put forward by an eminent group of scientists from various corners of the globe, saying, "GM crops have failed to deliver the promised benefits and are posing escalating problems on the farm. Transgenic contaminations in now widely acknowledged, being unavoidable and hence there can be no co-existence of GM and non-GM agriculture. Most importantly, GM crops have been proven unsafe. On the contrary, sufficient evidence has emerged verbose on serious safety concerns that if ignored could result in irreversible dame go health and environment. GM crops should be firmly rejected now. Further this panel said, "The consistent finding from independent research and on-farm surveys since 1999 is that genetically modified (GM) crops have failed to deliver the promised benefits of significantly increasing yields or educing herbicide and pesticide use. The instability of transgenic lines has plagued the industry from the beginning and this may be responsible for a string of major crop failures."

More recently 17 distinguished scientists from Europe, US, Canada and New Zeeland [47] wrote to the former Prime Minister of India, Dr. Monmohan Singh warning against "the unique risks of (GM crops) to food security, farming systems and bio-safety consideration which are ultimately would be fatally irreversible. This letter added "The GM transformation is highly mutagenic leading to disruption to hops plant's genetic structure/genome and metabolic profile which in turn paves to the way to disturbances in the biochemistry of the plant. This can lead to novel toxin or allergen production as well as reduced/altered nutrition quality.

This widely quoted letter added, "… the basic problem is that GM as employed in agriculture is conceptually flawed, crude imprecise and poorly controlled technology, this is incapable of generation plants that contain the required multiple coordinately regulated genes that wok in an integrated way to respond to environmental challenges …"

"… Gm has not increased yield potential. Yields from GM crops to date have been no better and in the case of GM soya have been consistently lower…. GM crops have led to vast increased in pesticide use not decreases and therefore reduction of agriculture pollution cannot be claimed. The toxicity of pesticides to human their ability to remain in the environment and accumulate in agricultural products requires the establishment of strict scientifically substantiated regulation for their safe application. In India, the rules for using pesticides are worked out together by the Union ministries for Agriculture and Health. Every year an approved "List of Chemicals and Biological Means for Controlling Pests, Plant diseases and Weeds Allowed to be used in Agriculture" is jointly issued by the ministries. Consequently, when employing pesticides it is necessary to adhere to the list approved for the current year and also abide by the instruction on the application of the pesticides compiled in strict conformity with the requirement adopted for the relevant substances.

In April 2009, the Union of Concerned Scientists (UCS) published a report "Failure to Yield" confirming that "after 20 years of research and 13 years of commercialization, GM crops have failed to increase yields" and that "traditional breeding outperforms genetic engineering hands down". There are thus enough studies to confirm the worldwide experience of overall poor performance and new hazards of GM crops. Ignoring all this, just at the time when news of heavy damage to Bt cotton in Punjab has poured in, high level efforts were launched to get the approval of a GM mustard variety. Obviously this attempt to push GM technology in food crops would become even more fatal due to the high health hazards of all Indians.

In his widely acclaimed book "Genetic Roulette" Jeffrey M, Smith has summarized the results of a lot of research on the health hazards of GM crops/food. He depicted, "Lab animals tested with GM foods had stunted growth, impaired immunes systems, bleeding stomachs, abnormal and potentially precancerous cell growth in the intestines, impaired blood cell development, misshapen cell structures in the liver, pancreas, and testicles, altered gene expression and cellular metabolism, liver and kidney lesion, partially atrophied lives, inflamed kidneys, less developed brains and testicles, enlarged livers, pancreases and intestines, reduced digestive enzymes, higher blood sugar, inflamed lung tissues, increased death rates and higher offspring mortality."

Even though a lot of scientific evidence on GM food crops was collected at the time of the wide debated on Bt-brinjal recently, unfortunately all these lesson are being unheard of and the Indian government appears to be geared up to go ahead with many GM crops without proper trials with special emphasis on GM mustard crop. This is emerging as one of the biggest threat to agriculture, environment and health [47].

In spite of all these novel endeavours and remarkable achievements by leaps and bounds with GM crops, several nefarious threats with heinous controversies engulfed this new technology silently. Though Prof. Borlaugh and other scientists are of opinion that the emergence of "eco-terrorist" fringe, who form a small but detrimental intruders, well-financed by anti-science zealots are tirelessly trying to constrain the pace of development and noble exploitation of plant biotechnology preventing over 10 billion farmers to pick up transgenic crops making the prices of seeds and technology both heftily escalated.

4. Conclusions:

Plant molecular farming has been shown to be a promising biotechnological approach; however, because this approach is novel, its efficacy may be disputed. Methods that facilitate plant cultivation under extremely controlled conditions should be developed for the subsequent stages of this process, as we move away from aseptic plant-cell cultures to non-aseptic conditions in which plants are grown traditionally or are grown hydroponically using compost. Plant molecular farming has significant potential for the development of medicinal products. With regard to the history of plant molecular farming, the current major focus is to accelerate the improvement of plant biotechnological procedures for the generation of new products, as well as conventional products. The most important challenges in this field are identifying new plant resources and optimizing protocols for producing high levels of recombinant proteins. The cryptic medicinal plant such as *Andrographis paniculata* [43] can be introduced as an impending candidate [44], while the genetic [45], and proteomic [46] analyses of the herb have both performed promising horizons for being subjected to plant molecular farming.

So now we can opine that the students and researchers in plant sciences are in the threshold of tremendous opportunity vis-à-vis some articulated manipulative threats. But they should not dislodge ourselves from the focus areas and must be adequately empowered and well-equipped to cater all these persisting demands for food, shelter and livelihood to all the famished faces of the receiving end in the 3rd world countries. Despite all these startling discoveries plant scientists must not forget the "THE MOST' challenge of the 21st century which still haunt our minds, the burgeoning food scarcity in the 3rd world countries. The position of the people in the low-income countries contrasts starkly with that in the developed countries. The world's population has increased from 2.5 billion to 6.1 billion in the last 50 years and it is unlikely to stabilize before 2100, by which time another 3 billion hungry stomachs will demand the leg-space to stand. Each night over 800 million populace go to the bed hungry and suffer from malnutrition and one-fifth of the human population (about 1.2 billion people) try to make both ends meet on an earning of less than a dollar per day. Plant scientists, researchers and future students must face this formidable challenge ahead and engage their minds to solve and formulate devices for more food in an environment-friendly manner. But we are hindered by several geo-political encumbrances. Prime agricultural land is being diverted to non-agricultural uses to meet the growing demands for housing, urbanization and industrialization. There is a desperate need to produce more food from less land with lesser water consumption with reduced agrochemical inputs. Scientists are convinced today that the required high yield/high quality/low cost/low ecotoxic crops can be delivered by the exploitation of techniques of plant biotechnology in molecular breeding strategies. The commercial adoption of transgenic crops by peasants has been one of the most rapid and successful cases of technology diffusion in the history of agriculture. Between 1996 and 2002, the area planted commercially with transgenic crops has increased from 1.7 million hectares to 587 million hectares. Some 6 million cultivators in 16 countries are benefitted by growing transgenic crops and more than a quarter of such crops are grown in developing countries including our India.

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