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Expanding the global horizon of engineered crops: Miles ahead to go

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Transformations in agriculture parallel to the advancements in civilizations have been a novel practice to enhance the productive creation of Mother Nature to nurture the society. Scientific advances of last century featuring identification of DNA, the development of molecular biology and its exploitation in life sciences have taken a lead. Progressions such as crop improvements through genetic modifications/genetic engineering/recombinant DNA technology are major milestones in this breakthrough technological revolution, offering promising hands for greater challenges in conventional breeding approaches of the 21st century. Introduction of recombinant DNA technology and its resultant as "Genetically modified (GM)", "Genetically engineered" or "Transgenic" crops accepted the challenge and delivered commercially viable herbicides, insects and viruses resistant entities, etc. On the global transgenic map, India is seen fortunate to be amongst top Bt-cotton producer, leading China by 11.6 million hectares in the year 2013 to 2014. India leads on Bt-cotton, many progressing in the pipeline, but with many controversial issues viz. Human and environmental safety, ethics, food security, commodity transfer and meddling with Mother Nature had always been raised as special concern before accepting this technology. This review projects era of transgenic, fostering the agricultural sector, breaking the challenges behind the acceptance of engineered crops and furthers its impact on growing agricultural economics.

Key words: Bt-cotton, biosafety, genetically modified (GM) crops, rDNA technology, transgenic, India.

INTRODUCTION

Plants transformed with desired favorable characteristics have been produced for thousands of years through conventional breeding. During this, the plants with desirable traits were selected and further propagated by the repeated sexual crossing process. This was a long process, taking decades to produce new varieties where it only works within the ambit of natural genetic barriers (Chetelat et al., 1995). With the entry of recombinant DNA technology, development of cloning and expression vectors supplemented with transformation and regeneration technology, in the late 70s, led to abolition of natural barrier of gene transfer. The onset of biotechnology, in the 1970s, known as Genetic Engineering technology accelerated in a drastic manner by just introducing few genes, which in addition, can also overcome the barriers of sexual incompatibility between plant species (Lemaux et al., 2008).

Introduction of transgenics or genetically modified (GM)

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is an entity generated using recombinant DNA technology that included gene of alien origin and had transformed the whole R&D and industrial picture. The technological know-how projects that a gene is expressed which is not native to the plant and their expression where the protein is encoded by the gene confers a particular trait or characteristic of that plant. The technology was found feasible for tribulations *viz.,* abiotic stresses like drought, extreme temperature or salinity and biotic stresses like insects and pathogens, that would normally prove detrimental to the survival of plants. It was also found to enhance the nutritional status of the plant, thereby adding value to existing ones etc. (James, 1998). Although, the acclimatization of plants to abiotic stress conditions is a complex and coordinated response involving hundreds of genes, these responses are also affected by interactions between different environmental factors and the developmental stage of the plant and could result in shortened life cycle, reduced or aborted seed production, or accelerated senescence (Mittler and Blumwald, 2010); these crops are suggested to provide a promising avenue to reduce yield losses, improve growth and provide a secured food supply for a growing population (Lemaux et al., 2008, 2009). Today, GM crops are being developed for the production of recombinant therapeutics and industrial products, such as MAbs, vaccines, plastics and bio-fuels.

The techniques, procedures, aspects and targets mentioned above seems very lucrative, but similar to every issue, it too has facets concerned with the biosafety, dealing with policies and procedures and norms adopted to ensure environmental safety (both biotic and abiotic components) during development and commercialization of transgenics (Rai and Prasanna, 2000). Under this issue, the following points are considered of high significance:

(a) Biosafety of unwanted gene flow and escape of engineered gene - where the actual prediction was found difficult with limited knowledge (Linder and Schmitt, 1994). Although the strategies to overcome the possibility of gene dispersal like isolation zone, trap crop and male sterility for no production of transgenic pollen have been proposed (Hernauld et al., 1993; Rai and Prasanna, 2000), the proponents of possibility of gene flow put their concern for unexpected ecological effects, invasiveness of transgenic traits and disturbance of biodiversity (Lemaux et al., 2008).

(b) Biosafety of antibiotic selection markers - which are used to select transformants *viz. nptII* gene conferring resistance against kanamycin. The potential threat was projected to be against human and animal consumption (Gressel et al*.,* 1992; Eady et al*.,* 1993). On the contrary, no harmful effect of such genes on human and animal health was demonstrated (Jan-Peter et al., 1992).

(c) Toxicity and allergenicity towards animals including humans - in 1989 claims surfaced that a nutritional supplement, L-tryptophan, used to treat insomnia, premenstrual syndrome, and depression, caused an epidemic in the U.S. which affected between 5,000 and 10,000 people and the number of deaths near 40; later, it was found to be not because of organism background but due to the procedure (Smith, 2003).

(d) The viral gene sequences (CaMV 35S promoter, etc.) used in plant genetic engineering have also been seen as potential risk factor which was rebutted by the scientific community as it is ubiquitous (Mae-Wan et al., 1999; Hodgson, 2000).

(e) Bt gene and its product were also projected as hazard but safety studies conducted specifically on native Bt proteins have shown that they do not have characteristics of food allergens or toxins (Mendelsohn et al., 2003).

(f) Creation of tolerant pathogens/weeds like "super-weeds". Indiscriminate and heavy use of herbicides, seasons after seasons is supposed to introduce the feature of herbicide tolerance in weeds making them "super-weeds" (Wrubel and Sheldon, 1993). This can be a major area of concern and improved agricultural practices can help to a great extent. Similarly generation of "super- viruses" (virulent to resistant varieties) are point of concern which may emerge through recombination (either by copy choice or cut and ligation), which may enhance pathogenicity. Instance of RNA recombination has been shown (Greene and Allison, 1994). However, such recombination between two viruses that would give rise to more new pathogenic strain, is less likely (Tabei et al., 1994).

(g) Unpredictable long term effects on health and biodiversity – as genes do not express in isolation, the expression syndrome in the long term may have unpredictable effects, the transgenic must be evaluated at more wider scale covering more physiological, morphological and genetic parameters within and beyond the target plants. It is more relevant because GM seeds have special characteristic of being living artifacts, which are able to reproduce but difficult to predict where to end up (Zoë, 2016).

The objections of scientists and academicians are colossal on all facets of GM crop development on both pros or cons side, but the most serious concern is for GM food crops with toxin genes which claims resistance against pathogens and pests, especially having Biotechnology background.

Although the level of potential of risk may be tremendous or negligible, the early evaluation of such entity becomes significantly high on safety marks. Hence to monitor such novelties and regulate them at various levels, efforts are being made worldwide. Considering the safety aspects of GM food, WHO along with FAO presented a report entitled, "Safety aspects of genetically modified foods of plant origin" (2000) considering most of the areas including approaches to the nutritional and food safety evaluation of genetically modified foods, nutrition-

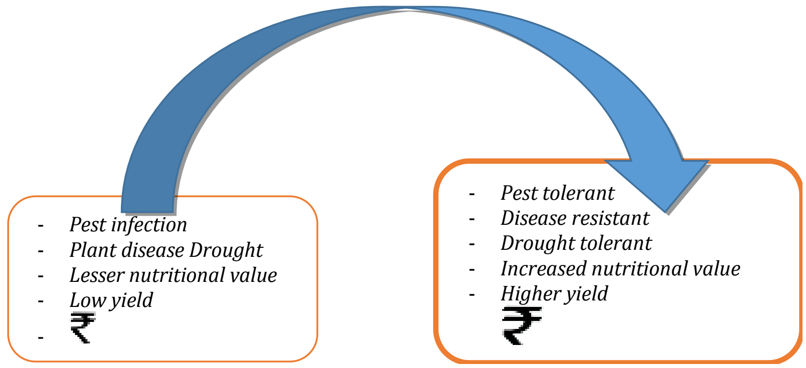

Figure 1. Impact of GM Crop on agricultural issues.

related issues, specific food safety issues covering safety assessment and allergenicity.

GE as effective agricultural feeder

India is an agriculture dominant country with 158 million hectares area which is a primary source of income for around 60% of the population. The country produces most of the crops to meet the requirement of food, fodder, fuel and other agricultural inputs. India is recognized as the largest producer of pulses, milk, tea and second largest producer of rice and wheat, fruits and vegetables, sugarcane and cotton. According to India Brand Equity Foundation, India is known as the 2nd largest agricultural land bearer in the world for its 157.35 million hectares of agricultural land. In FY2015, total food grain production in India was recorded at 252.68 million tonnes, which augmented to 253.16 million tonnes in FY16. Production of pulses was approx. 8.70 million tonnes. India also holds 2nd place as the largest producer of sugar, accounting for 14% of the global output. Being the 6th largest exporter of sugar, Indian accounts for 2.76% of the global exports. (India Brand Equity Foundation, Government of India, 2016).

Biotechnology has actively played a revolutionizing role in agriculture, thus transforming a consumers' life. Whether medicine or food or industrial products, expression of biotechnology application was found. Taking agriculture, for instance, the country faced a massive problem *viz.* pest and disease, drought tolerance and low nutritional value. Introduction of GM crops resolved such issues and transformed her from a net-importer country to net-exporter country of cotton thus increasing the smiley arc of farmers (Figure 1).

Today, GM crop is not a new name to India. The country welcomes commercial GM crops and made massive success, that is, the Bt-Cotton – a widely accepted crop which has created a success story for the Indian agriculture. According to The International Service for the Acquisition of Agri-biotech Applications (*ISAAA*), Bt-Cotton amplified its productivity in terms of area from 50,000 ha (2002) to 11 mn ha (2013). Successively, the farmers plotted around 11.6 million ha (2014) covering almost 96% of the total area. The country is well recognized to have world largest area under GM cotton, accounting for 1/3rd of global cotton (2013). To add to its achievements, a massive decrease of 85% was observed in insecticide sale as well, that is, US$160 mn (2004) to US$25 mn (2010). With such a humongous success, transgenic crops like pigeon pea, brinjal, potato, tomato, cabbage etc. are in pipeline and on experimental and evaluation phases. The only concern lies with human and environmental release and its impact assessment.

Downsizing pesticide usage

Since long, back cultivators have used pesticide as the only option left to safeguard their agricultural produce. The consumption of pesticides on global scenario is approximately 2 mn tonnes per annum of which 45% alone is consumed by Europe and 25% by USA, whereas the rest of the world consumes 25%. India is reported to share just 3.75% (De et al., 2014). GM has tremendously revolutionized this factor as well by decreasing the usage of pesticide globally; where cultivators sowing biotech crops (1996) have reduced it by 8.8%, that is, around 503 mn kg, which led to an overall reduction in the environmental footprint of biotech crops by 18.7% (Table 1).

Guarding research and field trails

In India, strict guidelines are structured for GMOs and products "Rules for the manufacture, use/import/export and storage of hazardous microorganisms/genetically engineered organisms or cells, 1989" set by Ministry of Environment and Forests (MoEF), Government of India under the Environment (Protection) Act (1986). These rules are implemented effectively by the Department of

Table 1. Impact on pesticide and herbicide use in the GM crop world (1996 to 2012).

Impact of changes in the use of herbicides and insecticides in GM crops globally, 1996-2012				
GM trait	Change in volume of AI used (mn kg)	Change in field EIQ impact (million field EIQ/ha units)	% change in AI use on GM crops	% change in environmental impact associated with herbicide and insecticide use on GM crops
HT Soybean	-4.7	-6,654	-0.2	-15.0
HT Maize	-203.2	-6,025	-9.8	-13.3
HT Canola	-15.0	-509	-16.7	-26.6
HT Cotton	-18.3	-460	-6.6	-9.0
IR Maize	-57.6	-2,215	-47.9	-45.1
IR Cotton	-205.4	-9,256	-25.6	-28.2
HT Sugar Beet	+1.3	-1	+29.3	-2.0
Total	-503.1	-25,121	-8.8	-18.7

HT = herbicide tolerant, IR = insect resistant, Ai = active ingredient, EIQ = environmental impact quotient. Environmental Impact Quotient (EIQ) - a universal indicator where the various environmental impacts of individual pesticides are integrated into a single field value per hectare. This EIQ value is multiplied by the amount of pesticide active ingredient (Ai) used per hectare to produce a field EIQ value. Environmental footprint: - Measure of the impact a product, process, operation, an individual or corporation places on the environment.
Source: Brookes and Barfoot (2014).

Biotechnology (DBT), Ministry of Environment and Forests (MoEF) and Ministry of Science and Technology and the State Governments along with six competent authorities mentioned in the Guidelines, Institutional Biosafety Committee prepared by DBT in association with BCIL, 2nd revised edition, May, 2011).

(a) Recombinant DNA Advisory Committee (RDAC);
(b) Review Committee on Genetic Manipulation (RCGM);
(c) Institutional Biosafety Committee (IBSC);
(d) Genetic Engineering Appraisal Committee (GEAC);
(e) State Biotechnology Coordination Committee (SBCC);
(f) District Level Committee (DLC).

The regulatory authorities function excellently on the axis of National Biotechnology Development Strategy to foster the country’s biotechnology revolution and bridge the gap with developed nations. To define the functionaries, RDAC acts in advisory where IBSC, RCGM, and GEAC are involved in major regulatory and project definitions. SBCC and DLC are made responsible for monitoring the various activities related to Genetically Modified Organisms in state/district level. RDAC, RCGM and GEAC are constituted at the central level by DBT and MoEF. IBSCs at organizations level, SBCC in all states, and DLCs in districts, wherever necessary. Out of the above, the IBSC is the nodal point for interaction within an organization for implementation of the biosafety regulatory framework. An IBSC is to be constituted by every organization engaged in research, handling and production activities related to GMOs, and each IBSC has a nominee appointed by DBT. The role of IBSCs is extremely important as it is a Statutory Committee that operates from premises of the respective organization. Functions of IBSCs have been elaborated in the *“Recombinant DNA Safety Guidelines, 1990”* and *“Revised guidelines for research in transgenic plants, 1998”* issued by the DBT.

The aim of these guidelines is to provide guidance to organizations having IBSCs or intending to set up the same in compliance with “Rules for the manufacture, use/import/export and storage of hazardous microorganisms/ genetically engineered organisms or cells, 1989” (hereinafter referred as Rules, 1989) notified by the Ministry of Environment and Forests (MoEF), Government of India under the Environment (Protection) Act, 1986. Being a statutory committee operating from premises of an organization, IBSC is in a position to carry out onsite evaluation, assessment and monitoring of adherence to the biosafety guidelines with overall oversight of the regulatory process, at the institutional level.

The ultimate aim is to promote research for beneficial objectives and keep monitoring and regulating the activities at various levels for safe and friendly future. At least agencies have been constituted as a step, with many more still ahead, e.g. setting norms for regulating the use/application of plants produced via genetic engineering or novel genome editing/testings (Thorben et al., 2016).

CURRENT STATUS OF *BT* TECHNOLOGY

By the end of the year 2013, *Bt*-crop plantation accounted for approximately 28.8 million hectares of land. The contribution (by 2012) to this acute transforming agriculture platform, by the developing nations like India, in adopting the GM crops, exceeded the statistics set by industrialized countries by a margin of 4% (James, 2012). This contribution comes from the unexpected domain of cultivators who were encouraged to adopt this thriving technology. 90% of such GM crops cultivators were actually resource-deprived, small scale

Table 2. Commercialized GE innovation (*Bt*–crops and its products) 1996 - 2013.

Bt – crop	Country
Cotton	United States of America (USA), India, Japan, Argentina, Australia, South Africa, Brazil, Myanmar, Burkina Faso, Canada, China, Colombia, Costa Rica, European Union (EU), , Mexico, New Zealand, Pakistan, Paraguay, Philippines, Singapore, , South Korea,
Maize	United States of America (USA), Australia, Brazil, Canada, Chile, China, Colombia, Egypt, El Salvador, EU, Honduras, Indonesia, Argentina, Japan, Malaysia, Mexico, New Zealand, Panama, Paraguay, Philippines, Russian Federation, Singapore, South Africa, South Korea, Switzerland, Taiwan, Thailand, Turkey, USA, Uruguay
Eggplant	Bangladesh
Tomato	USA, Canada, Chile,
Soybean	USA, , Brazil, Canada, China, Colombia, Uruguay, EU, Japan, Mexico, New Zealand, Paraguay, South Korea, Taiwan, Thailand, Argentina, Australia,
Potato	Australia, Canada, Japan, Mexico, New Zealand, Philippines, Russian Federation, South Korea, USA
Rice	Iran, China
Poplar	China

For the first 17 years of commercialization (1996 to 2012), benefits from insect resistant crops are valued at US$68.9 billion, 60% of the global value of biotech crops of US$116.9 billion; and for 2012 alone at US$12 billion, 64% of the global value of biotech crops of US$18.7. Source: ISAAA's GM Approval Database (http://www.isaaa.org/gmapprovaldatabase/)

farmers of developing countries. China and India (7.2 mn each) dominated the GM Crop domain with about 75% of farmers contributing together to this cultivation. ISAAA reports that the economics of cotton as a common GM crop in India and China – formerly stands more efficient (about 40% economical) in terms of average cost of production per hectare (James, 2013; Navarro and Hautea, 2014). Scientists envisage that this picture may take a drastic transformation with an affirmative style if more of such GE crops are stringently evaluated and approved for cultivation and consumption (Table 2).

CONCLUSION

The current scenario depicts that the area of crop cultivation is limited by edaphic and climatic traits. Thus, to expand, more crops need to be screened on various biotic and abiotic factors of losses.

GM crops, though faces controversies in many corners of the world, but have significantly revolutionized agriculture. Early '90s came with commercially viable GM technologies which also projected India as independent in terms of food aid or importer of food. Acceptance of GM crops worldwide was not an easy task, but challenges like pests, disease, drought and use of pesticide were major concerns and were mediated through GM intervention. It was a promising technology, which improved the economy of farmers as well as that of the country. Today, public and private sectors had jumped into this thriving technology with product galore. We say that the second green revolution is energizing with commercially viable crops like brinjal, cabbage, castor, cauliflower, groundnut, potato, tomato to name a few, with some running under trials. This blossoming technology is still thriving to enter into consumer's heart but that can only be a success with a deliberate involvement of research personnel, regulatory authorities and government fostering this industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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