

Recombinant DNA Technique: Tool to modify plant traits

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Submission : 22.08.2025

Acceptance : 19.08.2025

Publication : 30.12.2025



Abstract

Recombinant DNA refers to DNA molecules from two different species that are put into a host organism to create new genetic combinations useful in science, medicine, agriculture, and industry. Genetic modification entails incorporating DNA into an organism's genome as well as putting new DNA into plant cells in order to create a genetically modified plant. More effective and sustainable agriculture is dependent on plant varieties and cropping techniques that are more resistant to diseases, pests, and other environmental pressures. The world's population may exceed nine billion by 2050. To meet the requirements of such a large number of people in previous centuries, food production will need to expand at the same or higher rate. As a result, in recent decades, there has been a need to apply genetic methods to enhance crops. Transgenics can be used to develop plants with desired features and even greater yields. Transgenic breeding technologies provide new possibilities to breed for more resistant variants by utilising considerably larger genetic resources. Transgenesis, in essence, is a significant supplement to traditional plant breeding in that it allows for the focused manipulation of certain traits utilising genes from a variety of sources. Biotechnology has paved the way for the incorporation of transgenic crops into sustainable food production systems by bringing beneficial features into crops. Although these scientific achievements inaugurated a new age in agricultural production, broad adoption confronts a variety of obstacles owing to environmental, human health, and moral considerations. Crop genetic modification is expected to boost productivity and prosperity in sustainable agriculture methods. With the opportunity to ease global food crises, the proper use of transgenic crops has the potential to be more beneficial in coming future.

Keywords: Recombinant DNA, DNA techniques, Genetic modification, Transgenic, Biotechnology.

Introduction

Agriculture in the 21st century faces multiple challenges like erratic environmental condition, limited arable agricultural land and constantly growing human population worldwide. Factually, global population is expected to reach approximately 11 billion by the end of the century. These clearly indicate the necessity of quantifying crop production worldwide in order to fulfil present and future food security to ensure economic and social development. Climate change has seriously weakened agricultural productivity and increased the number of hungry people from 40 to 170 million (Altieri *et al.*, 2015). The supply of food, in terms of both its availability and its predictability over time, has been the primary concern for all human societies. Feeding the world population adequately would also mean producing the kinds of foods that are lacking to ensure nutrition security. In the developing country like India, agriculture is the primary source of livelihood for about 58 per cent of the population (IBEF, 2021) and approximately 70 per cent of the rural households depend on agriculture only. Agriculture is a crucial sector of Indian economy as it contributes about 20.19

per cent of GDP (DAC and FW Annual Report, 2020-21). The Green Revolution in the midst of 1960s, during which agriculture was converted into a modern industrial system by the adoption of technology, such as the use of high yielding variety (HYV) seeds, mechanised farm tools, irrigation facilities, pesticides and fertilizers which enables nation for food scarcity, now the country is facing second-generation problems, especially related to sustainability, nutrition, the adoption of new agricultural technologies. Food availability is not that reliable in India. The challenges to produce more and more for the growing population is becoming increasingly hard for a country of its size and economic growth. In India, the urban population was just 30 per cent in 2010, according to a UNDP assessment, but it is expected to increase to 40 per cent in 2030 and over 50 per cent by 2045. India's urban population is expected to expand from 3.5 billion to more than 6 billion by 2050. According to UNO - India, there are nearly 195 million undernourished people in India, which is a quarter of the world's hunger burden (Nutrition and Food Security - UN India, 2018). Also, roughly 43 per cent of children in India are chronically undernourished. India ranks 68 out of 113 major countries in

terms of food security index 2022. So, this shows that there is a need to improve the food production to feed growing population

Advancements in agriculture: A brief history

Agriculture, which is the foundation of human civilization, has undergone remarkable transformations throughout

history. From the earliest cultivation of crops to modern-day practices, agriculture has undergone significant advancements that have shaped the ways that we address agricultural challenges and improve crop characteristics. The brief timeline presented in

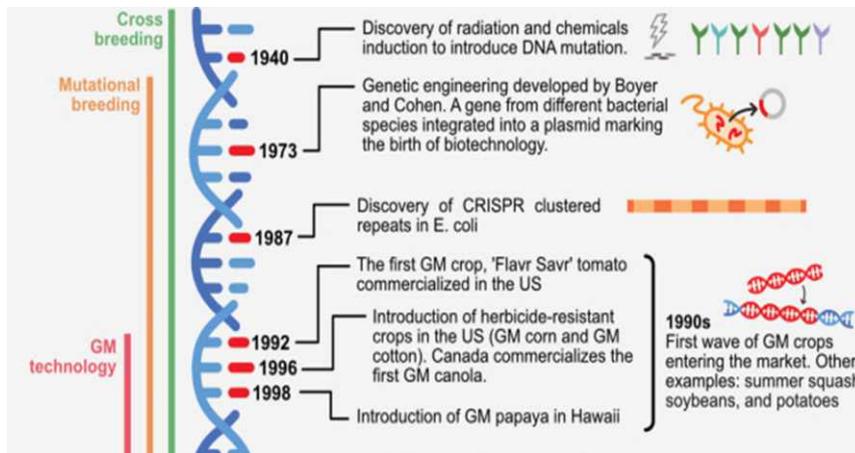


Fig. 1. Timeline of agricultural technology advancements throughout history

Fig. 1 highlights the key agricultural advancements throughout history, including various techniques that have greatly influenced crop improvement and agricultural productivity. To date, various conventional and modern genetic modification techniques, which include crossbreeding, induced mutagenesis, polyploidy, protoplast fusion, transgenesis, and genome editing, have been applied

in agriculture to produce healthier, tastier, and high-yielding crops (Fig. 2). These techniques represent the continuous human effort needed to manipulate plant characteristics to meet the demands of a growing population and evolving environmental conditions.

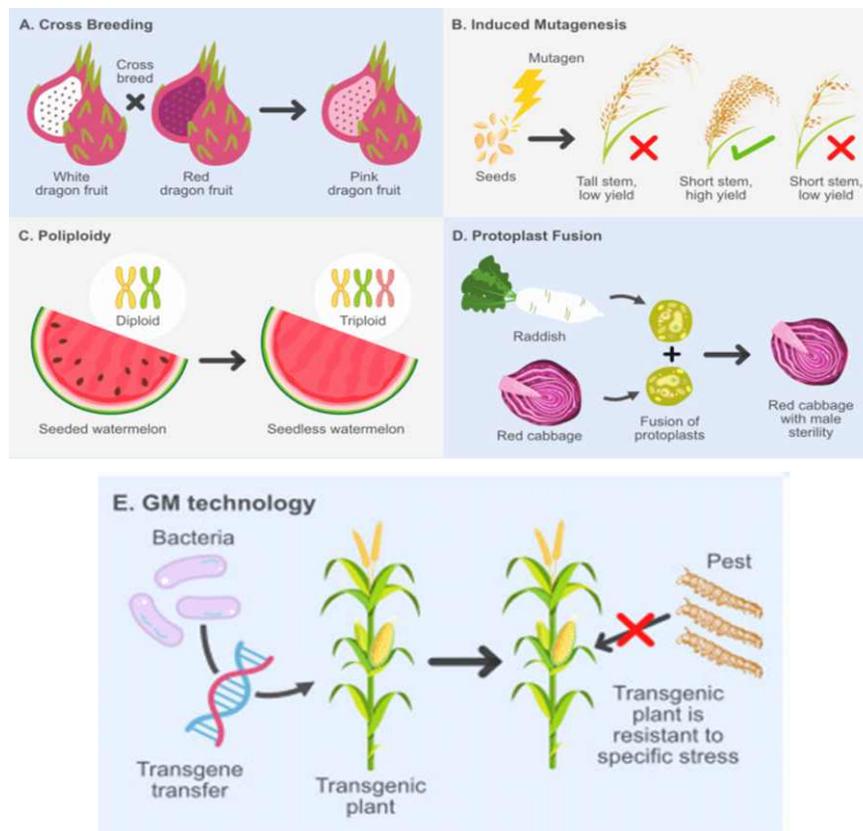


Fig. 2. Examples of plant breeding techniques in agriculture

(A) Crossbreeding involves selectively crossing different plant varieties to generate offspring with desired traits. (B) Induced mutagenesis employs mutagenic agents to induce controlled mutations, introducing new genetic variations. (C) Polyploidy manipulates plants to possess multiple sets of chromosomes, increasing genetic diversity. (D) Protoplast fusion merges the contents of plant cells from different species or varieties to create hybrid plants with desired traits. (E) GM technology (transgene transfer) involves introducing foreign genes into a target plant's genome to confer specific characteristics.

Crossbreeding, which is an age-old practice, involves the intentional mating of different plant varieties to obtain offspring with desirable traits. The approach has been instrumental in enhancing crop characteristics, such as yield, stress resistance, and nutritional content. However, crossbreeding is a time-consuming process that often requires multiple generations to achieve the desired results (Dou et al., 2022; Van Vu et al., 2022). Mutational breeding, which emerged in the mid-20th century, involves the induction of genetic mutations through radiation or chemical agents. This technique creates genetic variations that can lead to beneficial traits in crops. Mutational breeding has been successfully used to develop new crop varieties with improved characteristics, such as disease resistance and stress tolerance. However, the process of identifying and selecting desired mutations can be laborious, and unuseful mutations can occur (Lau et al., 2022).

Polyploidy doubles the number of chromosomes in an organism, resulting in altered gene expression and the potential enhancement of traits. This phenomenon can either occur naturally or be induced artificially through various techniques (Madani et al., 2021). In natural polyploidy, the genome of an organism undergoes duplication, leading to tetraploids (4n), hexaploids (6n), or higher multiples of the original chromosome number (te Beest *et al.*, 2012; Madlung, 2013). For instance, tetraploid wheat (*Triticum turgidum*) is the result of artificial crossbreeding between a diploid wheat and a diploid grass species, creating a hybrid that has four sets of chromosomes (Rosyara et al., 2019). Compared with its diploid ancestors, tetraploid wheat has larger grains and increased yield potential, making it a valuable crop for food production (Zeibig et al., 2022). On the other hand, artificially induced polyploidy involves the deliberate manipulation of an organism's genome, which is often achieved through the application of chemical or cell fusion techniques (Martinez-Fortun et al., 2022). The cultivated strawberry (*Fragaria* × *ananassa*) exemplifies

polyploidy studies, as it originated from wild progenitors to become the most widely cultivated fruit crop worldwide. The recent publication of its high-quality chromosome-scale octoploid genome has stimulated new research directions, including genetic variant characterization and investigation of molecular control mechanisms (Whitaker et al., 2020).

Protoplast fusion involves merging the protoplasts of different plants and individual plant cells that have undergone cell wall removal to create hybrids that have combined characteristics. During the 1980s and 1990s, protoplast fusion became increasingly popular as a valuable tool for genetic transformation due to the development of efficient methods, such as polyethylene glycol (PEG)-based transfection (Potrykus et al., 1985) and electroporation (Hauptmann et al., 1987; Negrutiu et al., 1987). Since that time, this technique has been widely used to generate novel germplasms and facilitate breeding programs, overcoming the challenges that are often encountered in conventional sexual crossing. Nevertheless, many challenges remain in the use of protoplasts for genetic improvement. One major limitation is the low efficiency of protoplast regeneration into whole plants (Reyna-Llorens et al., 2023). With the emergence of plant synthetic biology, protoplasts have the potential to accelerate genetic manipulation efforts, but the current techniques are still insufficient for realizing this potential.

The advent of GM technology in the 1990s revolutionized crop improvement. By 1994, the first commercially available GM crops were introduced, and their adoption quickly increased. As of 2014, more than 90% of the maize (*Zea mays*), soybean (*Glycine max*), and cotton (*Gossypium hirsutum*) plants cultivated in the United States were transgenic. GM techniques involve inserting or deleting specific genes, often from unrelated species, in an organism's genome to confer desired traits. This approach enables precise modification and rapid trait introduction, leading to increased crop productivity, pest resistance, and enhanced nutritional profiles. However, GM technology faces controversy and concerns over potential environmental and health impacts and other issues surrounding its public acceptance and regulation (Hamdan et al., 2022a).

DNA delivery methods

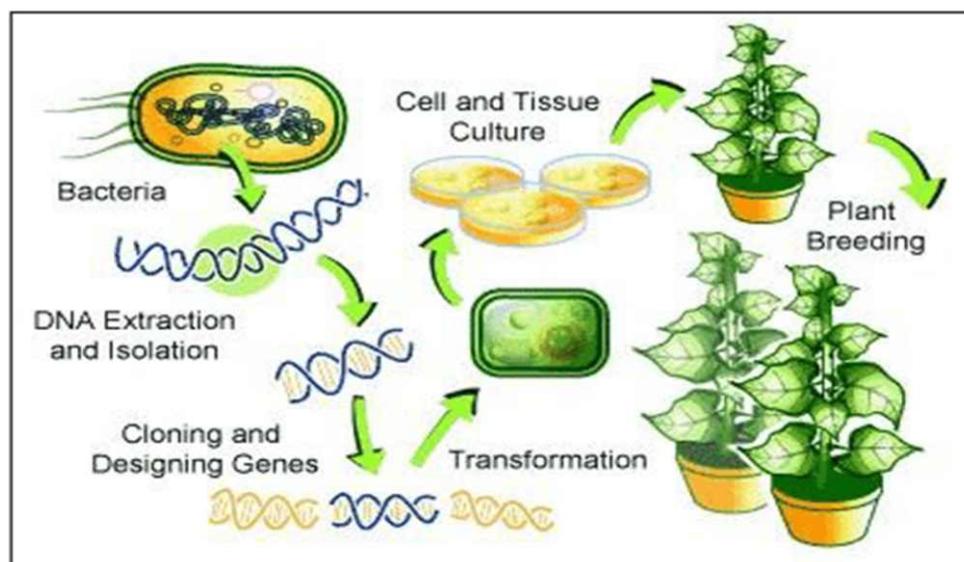
Transgenic technologies are aimed at incorporating specific genetic elements into plant cells to achieve desired traits. These two distinct approaches often employ similar DNA delivery methods for achieving the transfer of genetic material. A summary of the delivery methods employed in both technologies is presented in Table 1.

Table 1. Summary of delivery methods used in plant transformation

Transformation Techniques	Targeted tissues	Species	Delivery methods	Limitations
<i>Agrobacterium</i>	Root, shoot apical meristem, leaf, flower, hypocotyl, cotyledon	Monocots, dicotyledons	DNA	Host limitation
Biolistic/gene gun	Callus, shoot apical meristem, embryo, leaf	Monocots, dicotyledons	DNA, siRNA, miRNA, ribonucleo proteins	Cell damage, low integration efficiency, low expression levels
Protoplast fusion	Callus, shoot apical meristem, protoplast	Monocots, dicotyledons	DNA, siRNA, miRNA	Variable efficiency, requirement of cell fusion
Electroporation	Leaf, protoplast, meristem, pollen grain	Green algae, monocots, dicotyledons	DNA, siRNA, miRNA, proteins	Damage tissue, non-specific
Polyethylene glycol (PEG)	Protoplast	Monocots, dicotyledons	DNA, siRNA, miRNA	Cytotoxicity, low regeneration
Pollen pathway	tube pollen tube	Monocots, dicotyledons	DNA	Low efficiency
Nanoparticles	Leaf, protoplast	Monocots, dicotyledons	DNA, siRNA, miRNA	Low efficiency

Agrobacterium-mediated transformation is a widely used method for delivering genetic material into plant cells, in which *Agrobacterium tumefaciens* is used as a natural plant pathogen to transfer T-DNA into host plant genomes (Wang et al., 2023). The method is efficient and applicable to various plant species, including monocots and dicots, and has been successfully applied to crops such as maize, soybean, and cotton and to model plants such as *Arabidopsis thaliana* (Gelvin, 2003) and tobacco (Karlson et al., 2022). Challenges in *Agrobacterium*-mediated transformation include the

potential disruption of endogenous genes or regulatory elements during T-DNA integration, which can lead to unintended physiological effects in plants (Anjanappa and Gruissem, 2021). Further complexity arises from the varying transformation efficiencies that occur across plant species and genotypes, which necessitate tailored optimization strategies. In addition, the use of antibiotic selection markers for identifying transformed cells raises concerns about the potential dissemination of antibiotic resistance genes from genetically modified plants into the environment (Phillips, 2008).

Fig. 3. Gene Transfer through *Agrobacterium tumefaciens*

Biolistic particle bombardment, or gene gun-mediated transformation, is a versatile method for delivering exogenous DNA into plant cells without the need for specific plant-microbe interactions. High-velocity microprojectiles coated with the desired genetic material are propelled into plant tissue using a shockwave or electrical discharge. This technique is valuable because of its ability to transform a wide range of plant species, including recalcitrant species (Kikkert et al., 2005). However, these methods have limitations, such as random DNA integration and potential cell/tissue damage, which can affect their levels of transformation efficiency (Su et al., 2023).

Protoplast fusion involves the removal of cell walls from plant cells, resulting in the formation of protoplasts. These protoplasts can be fused together to create hybrid cells that incorporate genetic material from different sources (Yue et al., 2021). Protoplast fusion offers several advantages, including the ability to overcome species barriers and the capacity to facilitate the transfer of genetic material between distantly related plants (Reed and Bargmann, 2021). The technique has been successfully applied in various plant species, enabling the production of novel traits and the generation of valuable crop varieties. Its limitations include low fusion efficiency and the potential for chromosomal rearrangements or instability in the resulting hybrid cells (Yue et al., 2021).

Electroporation is applicable to a wide range of plant species, including both monocots and dicotyledons. By applying electric pulses, transient pores are created in the cell membrane that facilitates the efficient entry of exogenous molecules. While electroporation offers high delivery efficiency, it can also induce tissue damage and result in nonspecific uptake of genetic material (Gehl, 2023). Therefore, the optimization of pulse parameters and conditions when applying this method is critical for achieving specific and desirable genetic modifications while minimizing any adverse effects. Electroporation serves as a valuable tool in GM techniques to facilitate the introduction of desired genetic elements into plant cells and enable the exploration of targeted genetic modifications for agricultural applications (Jordan et al., 2008).

PEG is commonly used as a delivery method for introducing genetic material into plant protoplasts (Yang et al., 2023). PEG facilitates the uptake of DNA, siRNA, miRNA, and other molecules into protoplasts. The process involves incubating the protoplasts with a solution that contains both PEG and the desired genetic material. PEG functions by inducing cell membrane permeability and fusion, thus enabling genetic material to penetrate the protoplasts (Leh et al., 2024). The high molecular weight and hydrophilic nature of PEG create osmotic pressure that leads to a temporary disruption of the protoplast membrane (Mohd Amnan et al., 2021; Zhi et al., 2022). The disruption facilitates the entry of genetic material into the cell. However, PEG-mediated transformation can exhibit cytotoxic effects on cells and may result in low regeneration rates, thus limiting its overall efficiency (Mathur and Koncz, 1998).

The pollen tube pathway is used as a delivery method for introducing exogenous DNA into plant cells in both monocots and dicotyledons. Pollen grains are injected with foreign DNA and carried by growing pollen tubes as they elongate toward the ovary. However, the efficiency of this pathway is limited by the barriers encountered by these pollen tubes, competition between endogenous and exogenous DNA, and susceptibility to DNA degradation (Laforest and Nadakuduti, 2022). Researchers are exploring strategies for improving its efficiency by optimizing delivery techniques, enhancing DNA stability during pollen tube growth, and addressing competition with endogenous DNA.

Nanotechnology-based DNA delivery has emerged as a highly promising approach for the introduction of desired genetic material into plant cells in the use of GM technologies. The approach involves utilizing nanoparticles as carriers to protect and deliver DNA constructs (Ahmar et al., 2021). In recent developments, diverse nanoparticle carriers, including rosette nanotubes in wheat (Cho et al., 2020), sheet-like clay nanoparticles in tomato (Yong et al., 2021), and single-walled carbon nanotubes in oil palm (Zakaria et al., 2022), have been employed for pollen transformation.

Table 2. Examples and traits of GM crops with applications

Crop Type	Example	Genetic modification	Traits introduced	Applications	Reference
GM Crop	Bt Cotton	Bacillus thuringiensis (<i>Bt</i>) gene	Pest resistance	Reduced pesticide use	Vaeck <i>et al.</i> (1987)
	Roundup Ready Soybean	Glyphosate resistance gene	Herbicide tolerance	Simplified weed control	Zanatta <i>et al.</i> (2020)
	AquAdvantage Salmon	Growth hormone gene from another fish	Faster growth	Shorter production cycle	Hafsa <i>et al.</i> (2016)
	Golden Rice	Genes for beta-carotene production	Vitamin A production	Addressing vitamin A deficiency	Beyer <i>et al.</i> (2002)
	Bt Brinjal	Bt gene	Pest resistance	Reduced insecticide use	Shelton <i>et al.</i> (2018)
	HT Canola	Herbicide tolerance genes	Herbicide tolerance	Efficient weed control	Mayer and Furtan (1999)

Advantages of Genetically Modified Crops

GM Crops have several advantages which include:

Better than conventional breeding: Genetic Modification is one of the best methods to develop pest-resistant crops such as Bt crops.

It is faster to introduce the required traits than by the conventional breeding process.

It enables genetic modification in such a way that may not have been possible through conventional breeding methods, such as the introduction of a gene from different organisms....

Pest resistance: The Bt genes are toxic to many pests, thus eliminating the need for externally applied chemicals.

It reduces the need for environmentally damaging pesticides.

Also increases yield, saves farmers' money and reduces environmental pollution.

Virus resistance: Virus-resistant traits can be introduced into vulnerable plants that lack natural resistance. It helps to increase the productivity of crops.

Drought-resistant plants: Plants can be modified to express tolerance to drought hence, it reduces the use of groundwater in water screening area.

Herbicide tolerance: GM crops are engineered to be resistant to specific herbicides that can be used to control weeds. It reduces soil erosion because the weed removal process such as ploughing and tilling is not required for these crops. This also reduces soil, water and air pollution.

Sustainable agricultural practice: Genetically modified crops enable farmers to use more sustainable agricultural practices, such as no-till farming, which keeps the carbon within the soil rather than in the atmosphere.

Enhanced nutritional value: Food crops can be modified genetically to increase their nutritional value. This can be beneficial to health, and increase food security around the world

Status of GM crops in India and world

Approved Crop:

Bt cotton is the only GM crop approved for cultivation in India, covering over 90% of the country's cotton-growing area (around 11 million hectares).

Other GM Crops:

Research and field trials are underway for other crops like chickpea, pigeonpea, corn, and sugarcane.

GM Mustard:

The Environment Ministry gave a conditional go-ahead for commercial cultivation of GM mustard in 2022, but it hasn't taken off due to legal challenges in the Supreme Court.

Regulatory Framework:

The cultivation of GM crops in India is governed by the "Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms, Genetically Engineered Organisms or Cells" (Rules, 1989) under the Environment (Protection) Act, 1986.

Global:

Major GM Crops:

Soybean, maize, cotton, and canola are the most widely grown GM crops worldwide, with traits like herbicide tolerance and insect resistance.

Major Producers and Exporters:

The United States, Brazil, and Argentina are major producers and exporters of GM crops.

Market Growth:

The genetically modified crops market is experiencing strong growth, with a compound annual growth rate (CAGR) of 5.8%.

Recent Scenario of Transgenics

In spite of biosafety and environmental concerns, the transgenic technology has been a method of choice for rapid development of improved crop plants and stacking of multiple favourable traits. In the last 22 years, the global area of transgenic crops has increased significantly from 1.7 million hectare in 1996 to 191.7 million hectares in 2018, *i.e.*, around 113-fold increases (ISAAA 2018). There were 95.9 million hectares (50 per cent) of transgenic soybean, 58.9 million hectares (31 per cent) of transgenic maize, 24.9 million hectares (13 per cent) of transgenic cotton, 10.1 million hectares (5.3 per cent) of transgenic canola and 1.9 million hectares of other transgenic crops (ISAAA 2018). In 2017–18, these crops were planted by approximately 17 Mio. farmers in 26 countries and their estimated global market value were US\$18.2 billion (ISAAA 2018). The major traits, for which transgenic crops have been developed and approved for commercialization, include herbicide tolerance (HT), insect resistance (IR), disease resistance, abiotic stress tolerance and nutritional enhancement (Kumar *et al.*, 2020). In India to meet the requirements of growing population utilization of transgenic crops is not being recognized at present. This is due to the result of concerns raised by the public and the critics about their applications and release into the environment. These include effect on human health and environment, biosafety, world trade monopolies, trustworthiness of public institutions, integrity of regulatory agencies, loss of individual choice, and ethics as well as skepticism about the real potential of the genetically modified plants, and so on. According to International Services for the Acquisition of Agri Biotech Applications (ISAAA) India has the fourth largest area planted under transgenic crops, a total 11.6 million hectares (mh) was under transgenic plantation in year 2014, only after, Brazil (42.2 mh), Argentina (24.3 mh) and USA (73.1 mh). Currently across the world approximately 181.1 mh areas was used for the transgenic crop plantation. In year 2009, India's Maharashtra Hybrid Seeds Company (Mahyco) and US based company Monsanto with has developed Bt egg plant (*Solanum melongena*) by inserting a crystal gene (*CryIAc*) from *B. thuringiensis* (Krattiger, 2010; Cotter, 2011). But still the Indian Government has imposed a moratorium on its release after its commercialization due to the public resentment (Report of the Expert Committee-II on Bt Brinjal Event EE-1, 2009).

Despite of various advantages the use of transgenic plants for human welfare has been restricted owing to various concerns raised by the public and the critics. These concerns are divided into different categories, namely, environmental, health, nutritional, ecological, socioeconomic, and ethical concerns. Certain public groups, including religious bodies,

find it very unethical or inhumane to introduce human or animal genes into plants (Whiteman, 2000). There is a potential risk that the GM plants may hybridize (or cross-breed) with sexually compatible wild-type species. This genetic exchange is possible due to wind pollination, biotic pollination or seed dispersal.

Acceptability of Transgenic Products: Genetic Stability Genetically modified crops such as maize, cotton, soybean and canola, containing biotechnology derived agronomic traits, have been rapidly adopted by growers around the world over the past 25 years (Brookes *et al.*, 2020). The majority of these crops express novel proteins and have undergone pre-market regulatory assessments prior to product authorization and commercialization.

To properly conduct a regulatory assessment, the safety of the newly expressed protein is integral (Delaney *et al.*, 2008). Along with in depth characterization of the event at the molecular level and aspects of its phenotypic/agronomic performance. A molecular characterization of the event is undertaken, which provides information on the structure and expression of the inserted DNA and on the stability of the intended trait(s) encoded by this DNA region. The following points are routinely addressed: 1) genetic stability of the (trans) gene(s) and the integration locus; 2) inheritance pattern of the event; and 3) stability of expression at the transcript (required only in a few geographies), and protein level across multiple generations. These assessment points are addressed by multiple analytical approaches and comparable guidance is provided for such studies by different regulatory bodies across the world (Privalle *et al.*, 2023).

Conclusion and Future Prospectus

It is acknowledged that the world will face a number of serious challenges if development is to proceed on a sustainable pathway. Indeed, many people in the world still live in extreme poverty and are without adequate nutrition, health and education it has been estimated that the requirement for food will double by 2050. Climate change threatens to reduce productivity in many regions. To meet these challenges humankind is requiring to adapt the available options and tools. transgenic crops can contribute to international targets and goals related to sustainable development. Transgenic technology has contributed towards development of crop varieties with enhanced yield, resistance to biotic and abiotic stresses, and enhanced food quality. Further, estimates also suggest that adoption of the transgenic technology has helped in reducing the use of pesticides and insecticides, decreasing environmental footprint and increasing farmer income. To address some of the major concerns associated with transgenic crops, new alternative techniques, such as cisgenesis, intragenesis and

most recently, genome editing, are being used to develop improved crop plants. Since a subset of the crops developed using genome editing techniques would be quite similar to the conventionally bred plants, it is hoped that such genome-edited crops might be granted faster regulatory approval which should lead to their widespread adoption in cultivation. As the world population continues to rise, the evidence reviewed here suggests it would be unwise to ignore transgenic crops as one of the tools that can help meet aspirations for increasingly sustainable global development.

Source of Support: Nil

Conflict of Interest: Nil

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