SCIENCE BEHIND TRANSGENIC CROP TECHNOLOGY

Abstract

Transgenic technology process begins with isolating specific genes responsible for desired traits, followed by their insertion into the target organism's DNA using various techniques like gene guns or bacterial vectors. This precise manipulation allows for the expression of novel traits, such as increased resistance to pests, disease and enhanced nutritional value. Researcher's analyses gene functions and their interactions to meticulously ensure the precise insertion and expression of genes. Furthermore, in India, regulatory authorities rigorously evaluate transgenic crops to ensure minimal environmental impact and no health risks to the public. Transgenic technology presents solutions to global challenges, including addressing food security, treating diseases, and establishing sustainable production methods. The continuous scientific advancements driving transgenic technology offer promising pathways for both scientific exploration and societal progress.

Keywords: Transgenic technology, Gene transfer method, GMO, Regulatory mechanism, misconception of transgenic crop.

Authors

Premkumar Adhimoolam
Department of Plant Breeding and Genetics
Pandit Jawaharlal Nehru College of Agriculture and Research Institute
Karaikal, Puducherry, India

K. ManojKumar
Department of Plant Breeding and Genetics
Pandit Jawaharlal Nehru College of Agriculture and Research Institute
Karaikal, Puducherry, India

J. Karthick
Department of Plant Breeding and Genetics
Pandit Jawaharlal Nehru College of Agriculture and Research Institute
Karaikal, Puducherry, India

S. Nadaradjan
Department of Plant Breeding and Genetics
Pandit Jawaharlal Nehru College of Agriculture and Research Institute
Karaikal, Puducherry, India

I. INTRODUCTION

During the early days, human began selecting desirable plants for their survival and development without aid of scientific knowledge. Unfortunately, a substantial amount of valuable genetic resource was neglected from selection resulting to bottleneck in domestication. Throughout the course of human history, various scientific personalities have dedicated themselves to understanding the mystery of trait inheritance; however, a significant breakthrough in this field occurred in 1865 when G. Mendel conducted a brilliant experiment on peas, leading to the birth of Genetics. As a result, breeders have developed various plant varieties using the conventional plant breeding strategies with objective to enhance yield, stability of performance *i.e.*, resistant to pest, disease and desirable quality attributes for consumer. The gene of interest can be sourced from the primary gene pool, secondary gene pool and tertiary gene pool. In the primary gene pool, the crossing program is relatively straightforward and convenient; whereas in case of secondary and tertiary gene pool, different strategies are required due to the occurrence of infertile or weak hybrids. In such cases, the utilization of embryo rescue and chromosome engineering becomes necessary to facilitate the transfer of desired genes, but a major limitation of this pool is that it restricts the opportunity for gene transfer solely within the same crop species, consequently gradually diminishing the options for genetic improvement. To expand the opportunities for genetic improvement, the concept of transgenic crops technology emerged and this is viable technology enables the transfer of gene of interest from any living organism other than crops which broadens the possibilities for genetic enhancement of crops not possible in conventional plant breeding strategies. This chapter deals with science behind transgenic technology, advantage and disadvantage, application and its drawbacks in details.

II. ORIGIN OF BT (TRANSGENIC) TECHNOLOGY

During the early 1900's Japanese scientist Shigetane Ishiwatari help unravel the cause of devasting "sotto disease" that caused widespread destruction of silkworm population. Ishiwatari isolated the bacterium responsible for the disease which later recognized as *Bacillus thuringiensis*. In another significant development, Berliner made a key discovery in 1911 by isolating a bacterium from the Mediterranean flour moth and name *thuringiensis* after the German town Thuringia where moth had been discovered. In 1915, Berliner reported the presence of crystal within Bt, but its significance was not fully realized until decade after the discovery. This bacterium was initially utilized to combat the European corn borer, marking a revolutionary event. This monumental breakthrough led to introduction of ground breaking Bt-based pesticide Sporine which debuted in France. Since that transformative milestone, Bt based bio-pesticide have emerged as a prominent and extensive strategy for pest control in the realm of organic agricultural practices. However, the utilization of Bt-based biopesticides, such as spray formulations, encountered certain drawbacks. They were vulnerable to rapid wash-off by rain and degradation under the sun's UV rays. Additionally, a limited range of available Bt strains exhibited toxicity exclusively towards lepidopteran larvae, leaving other insect species unaffected. Some insects also took up residence within the plant or underground, rendering them inaccessible to sprays. These limitations hindered the widespread use of Bt-based biopesticides.

In 1950, researchers achieved a groundbreaking milestone by uncovering the insecticidal activities of crystal proteins against lepidopteran insects. The pivotal discovery sparked enthusiasm among scientist to unravel crystal structure, biochemistry and

comprehensive mechanism of action of Bt. Comprehended extensive research on Bt (Genetically Modified Crops, GMO) on various crops commenced with utmost dedication by both public and private sectors.

III. METHODS FOR TRANSFERRING FOREIGN GENES INTO FIELD CROPS

Scientists have made significant advancements in discovering various methods to transfer genes of interest into field crops. These methods include electroporation, microinjection, chemical-mediated gene transfer, liposome-mediated gene transfer, agrobacterium-mediated transfer, and particle bombardment. However, among these strategies, agrobacterium-mediated transfer and particle bombardment have emerged as the most prevalent and widely employed methods for successful gene transfer into field crops.

1. **Agrobacterium Mediated Gene Transfer Method:** *Agrobacterium tumefaciens*, a gram-negative bacterium, possesses a unique ability to induce the formation of crown gall tissue in plants specifically in response to the plants secreting the compound, Acetosyringone. This intriguing phenomenon is primarily attributed to the presence of the Ti plasmid, which contains three genes responsible for tumor induction. The Ti plasmid exists as an independent circular DNA molecule capable of replicating within Agrobacterium cells. In the field of biotechnology, scientists utilize a method in which they replace the T-DNA region of the Ti plasmid with a gene of interest, along with suitable selectable markers. The resulting modified plasmid contains autonomous replication capability, making it capable of replicating independently within host cells. Furthermore, it offers ease of isolation and purification, allowing for efficient extraction and purification of the plasmid. The modified vector also demonstrates ease of introduction into host cells, enabling successful gene transfer. It incorporates a suitable marker gene that facilitates the selection and identification of transformed cells. Moreover, it includes a unique target site and recognition site for various restriction enzymes, enabling precise genetic manipulation. Additionally, the vector provides a multiple cloning site, facilitating the insertion of diverse DNA fragments. The schematic representation of the vector can be illustrated as follows:

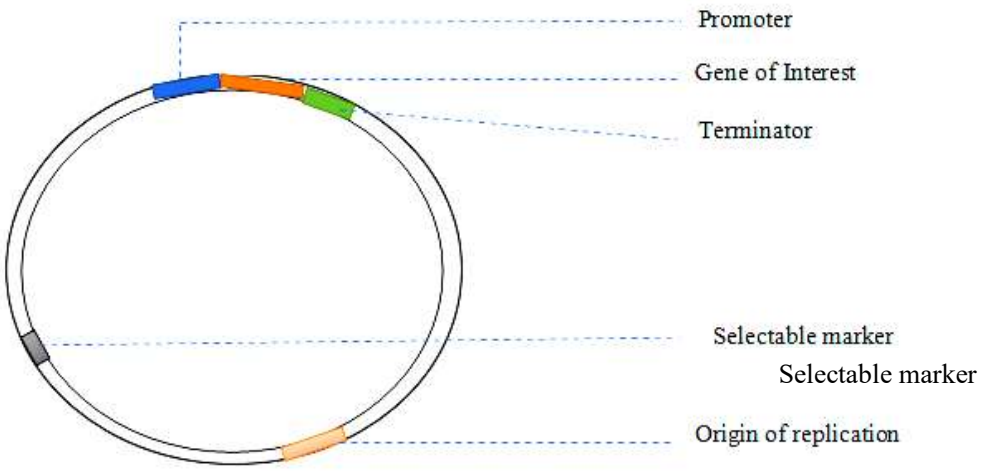


Figure 1: Schematic representation of vector

- **Protocol for Agrobacterium-Mediated Transfer into Field Crops:** The process begins by isolating the gene of interest from any organism, followed by constructing an expression cassette in which the gene of interest is positioned between promoter and terminator sequences. This cassette not only facilitates gene expression but also incorporates marker genes to assist in selecting transformed plants by monitoring the presence of introduced genes within the host plant.

  The next step involves inserting the expression cassette into a vector that contains the T-DNA region using restriction nucleases and ligases. Subsequently, the expression vector is transformed into Agrobacterium. In parallel, explants from the plant (which can be any part of the plant) are obtained and subjected to a brief period of co-cultivation with Agrobacterium after being wounded using Acetosyringone. This process facilitates Agrobacterium infection within the explants. The transformed explants are then cultivated in the presence of a selective antibiotic, which allows only positive transformants to survive. Positive transformants are identified through PCR analysis, and their transformation is further confirmed through Southern hybridization. Southern hybridization reveals the site of gene integration and determines the copy number of the desired gene. The transformed explants are carefully studied and subsequently progress to subsequent steps, ultimately leading to the stage of commercial cultivation.

2. **Particle Bombardment Method:** The particle bombardment method, also known as particle gun method, biolistic process, microprojectile bombardment, or particle acceleration, was developed by John Sanford *et al*., in the United States in 1987. This technique is used to introduce foreign genes into cells, providing an alternative to agrobacterium-mediated vector technologies that are limited to certain plant species and show insufficient response in crops like rice, wheat, corn, sorghum, chickpea, and pigeonpea. The principle of this technology involves accelerating DNA-coated microscopic particles (microcarriers) at high-speed using helium gas within a vacuum. The apparatus consists of a chamber connected to an outlet that creates a vacuum. At the top, a cylinder is temporarily sealed off from the rest of the chamber by a plastic rupture disk.

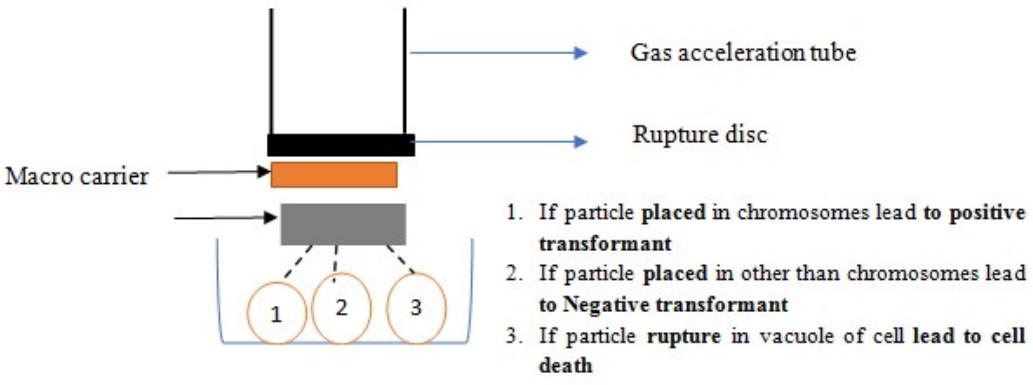


Figure 2: Particle bombardment method; 1, 2 and 3 indicates possibilities during acceleration into target cells.

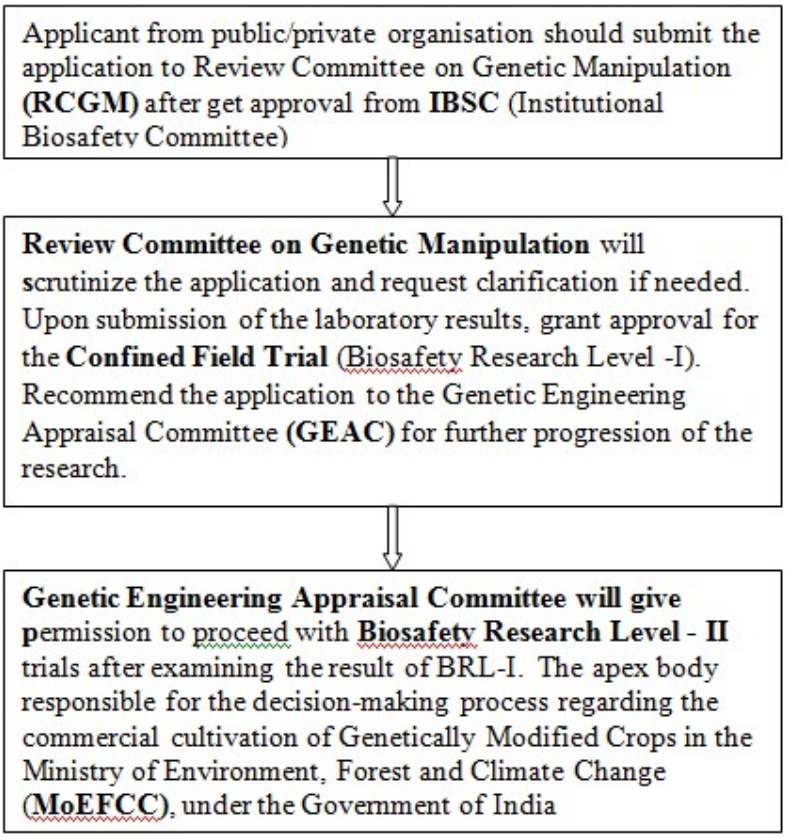
Helium gas flows into the cylinder, and a plastic microcarrier containing DNA-coated tungsten particles (coated microprojectiles) is positioned near the rupture disk, as depicted in Figure 1. For genetic transformation, foreign DNA is coated onto the surface of micron-sized tungsten or gold particles through precipitation with calcium chloride and spermidine. The DNA to be used for transformation is coated onto gold or tungsten particles (1–2 μm) as carriers. When the pressure inside the cylinder exceeds the bursting point of the plastic disk, it ruptures. Helium shock waves propel the plastic microcarrier containing DNA-coated micropellet into the target cells (cell suspensions, callus cultures, or tissues). The accelerated microprojectiles penetrate the plant cell walls and membranes; as the microprojectiles enter the cells, subsequently incorporating into the plant's chromosomal DNA. The transformed cells are then regenerated on nutrient media and the selected plants are subsequently analysed for the expression of foreign DNA either through PCR or southern hybridization methods.

IV. WHO APPROVES GENETICALLY MODIFIED CROPS IN INDIA

The applicant, whether from a public or private organization, will submit a research proposal related to genetically modified (GM) research to the Review Committee on Genetic Manipulation (RCGM) after obtaining approval from the Institutional Biosafety Committee (ISBC). The ISBC is a committee established by each institution and consists of the institution's head, a DNA manipulation expert, and a nominee from the Department of Biotechnology (DBT). The committee's responsibility is to verify that the research proposal complies with the guidelines outlined in the Environmental Protection Act of 1986 and the Rules for the Manufacture, Use/Import/Export & Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells of 1989.

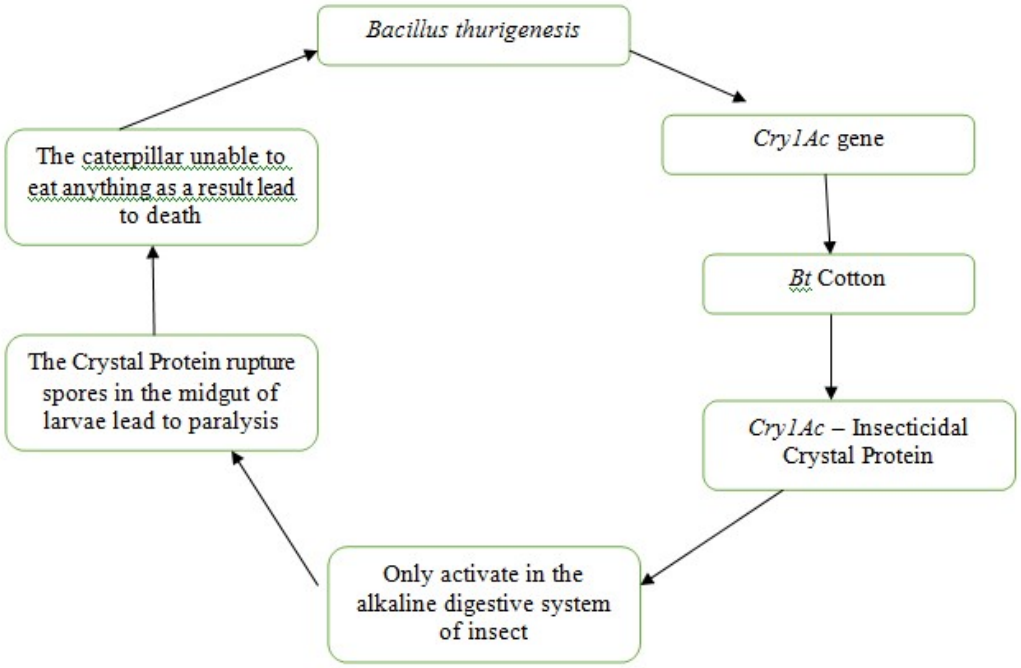
Once the application satisfies all the prescribed norms, the ISBC forwards the application to the RCGM. The RCGM reviews the application with the help of experts in the committee. If any clarifications are required, queries will be sent to the applicant; once the RCGM is satisfied, it grants approval to carry out the research. Upon successful completion of the research, the applicant submits the results of the laboratory experiments and seeks approval to conduct a Confined Field Trial (Biosafety Research Level Trial I). The RCGM thoroughly examines the submitted results and, if satisfied, forwards them to the Genetic Engineering Appraisal Committee (GEAC). The GEAC, which operates under the Ministry of Forest and Environment, Government of India, scrutinizes the data from the Biosafety Research Level I trial and, if deemed satisfactory, allows for the conduct of a Biosafety Research Level II field trial.

Ultimately, the GEAC decides on the commercial cultivation of the new GM product after ensuring that it is safe for human consumption, does not pose any harmful effects or allergies, and is environmentally friendly. The flowchart describes the sequential steps involved in the application submission, scrutiny, and approval for commercial cultivation as follows:



V. SCIENCE OF BT COTTON

Cotton is a highly demanded commercial crop in the textile industry and is extensively cultivated by farmers in India. However, cotton cultivation faces significant challenges due to destructive pests such as the American bollworm and pink bollworm, which cause substantial losses to growers. These polyphagous insects predominantly attack cotton during the fruiting period, damaging the lint and impacting cotton prices. To combat these pests, farmers have traditionally relied on excessive insecticide use, resulting in environmental pollution and increased cultivation costs. Fortunately, biotechnology has brought about a significant breakthrough in the form of the *Cry1Ac* gene found in *Bacillus thuringiensis*. This gene has been successfully transferred into cotton, resulting in the development of Bt cotton. Bt cotton offers protection against pink bollworm and American bollworm attacks. As a result, farmers can mitigate the pest infestation without resorting to excessive insecticide application. The accompanying picture illustrates the process involved in isolating the gene, introgressing it into the crop, and the resulting impact on caterpillars.



Status of BT cotton in India:

The Government of India authorized the commercial cultivation of Bt cotton in 2002. Despite facing challenges related to misinformation, this technology has earned the trust of farmers, researchers, and policymakers due to the remarkable benefits offered by genetically engineered hybrid seeds. The positive outcomes witnessed in the field include effective pest control, leading to higher yields and improved farm incomes.

The extent of adoption is evident from the fact that in the 2019-20 season, approximately 11.7 million hectares (93.6 percent) of the total 12.5 million hectares of cotton cultivation in India were planted with Bt cotton seeds. Over the past decade, cotton yields in the country have significantly increased compared to non-Bt varieties, with less harm to the environment (Directorate of Economics and Statistics, Ministry of Agriculture, Government of India). Indian agriculture now necessitates new technologies to ensure the competitiveness of its farmers on a global scale.

VI. REALITY OF BT TECHNOLOGY

1. **The Fact of the GMO in Related to Human Health:** Contrary to the misconception, GMO foods undergo rigorous laboratory tests, field trials, and safety assessments for human consumption, demonstrating no negative impacts on health or the environment. In countries like India, the regulatory system involves comprehensive scrutiny by expert committees, including institution heads, scientists, and medical professionals, from the initiation of experiments to commercial cultivation. Only after thorough evaluation of all reports are GMOs approved for cultivation. A notable example in India is Bt cotton,

which has significantly reduced the need for insecticides/pesticides and minimized environmental damage

2. **GMO Crops are safe to Environment:** GMO crops have proven to have a positive influence on the environment, making significant contributions to sustainable farming practices. One notable impact is the reduction in chemical pesticide usage. Studies have shown that GMO crops have decreased chemical pesticide and it reduces the environmental pollution.

3. **Impact of GMOs on Cotton Species Conservation:** Bt Cotton technology has been developed specifically for the *Gossypium hirsutum* species, which is not compatible with *G. arboreum* and *G. herbaceum*. This targeted approach ensures that the genetic modifications introduced through Bt Cotton technology remain confined to the intended species and do not cross breed with other native cotton varieties in India. Furthermore, it is important to note that no wild relatives of cotton have their origin in India. As a result, concerns regarding the drifting of the genetically modified genes to wild relatives of the crop are eliminated. The absence of wild relatives in the country mitigates the risk of gene flow from Bt Cotton to these species, ensuring that the modifications are contained within the cultivated cotton varieties.

4. **Ensuring the Safety of BT Crops in the Animal and Human Food Chain:** The safety of Bt cotton in relation to food consumption has undergone rigorous evaluation at the authenticated research laboratory in India. Through a comprehensive trial, laboratories conducted thorough assessments of seeds, oil, and cake derived from Bt cotton. The conclusive findings of their reports affirm that Bt cotton poses no harm to mammals, ensuring its absolute safety in the food chain.

VII. CONCLUSION

In India, with the human population continuously increasing, the need to enhance crop yields within limited resources such as land, labour, and water becomes ever more crucial. To meet this challenge, the adoption of advanced agricultural technologies becomes imperative. Looking ahead, the adoption of advanced agricultural technologies is anticipated to play a vital role in achieving a hunger free situation in the future.

REFERENCE

[1] Abbas, M.S.T. (2018). Genetically engineered (modified) crops (*Bacillus thuringiensis* crops) and the world controversy on their safety. *Egyptian Journal of Biological Pest Control*, 28, 52.
[2] Ahuja, V. (2018). Regulation of emerging gene technologies in India. *BMC Proceedings*, 12(8), 14.
[3] Abdul Aziz M., Brini, F., Rouached, H. and Masmoudi, K. (2022). Genetically engineered crops for sustainably enhanced food production systems. *Frontier Plant Science,* 13, 1027828.
[4] Altpeter, F., Baisakh, N., Beachy, R., Bock, R., Capell, T., Christou, P., Daniell, H., Datta, K., Datta, S., Dix, P.J., Fauquet, C., Huang, N., Kohli, A., Mooibroek, H., Nicholson, L., Nguyen, T.T., Nugent, G., Raemakers, K., Romano, A., Somers, D. A., Stoger, E., Taylor, N. & Visser, R. (2005). Particle bombardment and the genetic enhancement of crops: myths and realities. *Molecular Breeding*, 15, 305–327
[5] Chew, Y.C., Abdullah, W. M. A. N. W., Kok, D, A., Ong-Abdullah, J., Mahmood, M., & Lai, K.S. (2019). Development of an efficient particle bombardment transformation system for the endemic orchid, *Phalaenopsis bellina*. *Sains Malaysiana*, 48(9), 1867–1877

[6] Choudhary, B., Gheysen, G., Buysse, J., Meer, P. V. D. & Burssens, S. (2014). Regulatory options for genetically modified crops in India. *Plant Biotechnology Journal*, 12,135–146
[7] Gatehouse, J. A. (2008). Biotechnological prospects for engineering insectresistant plants. *Plant Physiology*, 146, 881-887.
[8] Hagio, T. (1998). Optimizing the Particle Bombardment Method for Efficient Genetic Transformation. *Japan Agricultural Research Quarterly*, 32, 239-247
[9] Handbook for Food Safety Officials Genetically Modified Foods: Safety Assessment and Regulations. (2019). Ministry of Environment Forest and Climate Change, Government of India and Biotech Consortium India Limited New Delhi under the UNEP/GEF supported Phase II capacity building project on Biosafety.
[10] Khadi, B.M., Kulkarni, V.N., Ansingkar, A. S., Singh, V. V. & Gotmore, V. (2003). Gene pool concept for diploid Gene pool concept for diploid cultivated cotton cultivated cotton. *World Cotton Research Conference-3, Cape Town - South Africa*, 214-225
[11] Keshavareddy, G., Kumar, A. R. V. & Ramu, V.S. (2018). Methods of Plant Transformation- A Review. *International Journal of Current Microbiology and Applied Sciences*. 7(7): 2656-2668
[12] Mookkam, M. (2018). Particle bombardment – mediated gene transfer and GFP transient expression in *Seteria viridis. Plant Signaling and Behavior*, 13(4), e1441657.
[13] Oliver, M.J. (2014). Why we need GMO crops in agriculture. *Missouri Medicine*, 111(6), 492-507.
[14] Vega Rodríguez, A., Rodríguez-Oramas, C., Sanjuán Velázquez, E., Hardisson de la Torre, A., Rubio Armendáriz, C., Carrascosa Iruzubieta, C. (2022). Myths and Realities about Genetically Modified Food: A Risk-Benefit Analysis. *Applied Science*, 2022, 12, 2861
[15] Subhasmita, S. & Pradeepkumarap N. (2022). Concept of Gene Pool and Distant Hybridization in Vegetable Crops. *International Journal of Current Microbiology and Applied Sciences*, 11(05), 148-159
[16] Sanahuja, G., Banakar, R., Twyman, R.M., Capell, T. & Christou, P. (2011). Bacillus thuringiensis: a century of research, development and commercial applications. *Plant Biotechnology Journal*, 9, 283–300.
[17] Shukla, M., Al-Busaidi, K. T., Trivedi, M. & Tiwaria, R. K. (2018). Status of research, regulations and challenges for genetically modified crops in India. *GM Crops & Food*, 9:173–188
[18] Taylor, N. J. & Fauquet, C. M. (2002). Microparticle bombardment as a tool in plant science and agricultural biotechnology. *DNA Cell Biology*. 21(12), 963-977.

