The Pharma Innovation Journal 2022; SP-11(6): 753-758

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 753-758
© 2022 TPI
www.thepharmajournal.com
Received: 11-04-2022
Accepted: 14-05-2022

Kondeti Sai Sumanth
M.Sc Student, Department of Genetics and Plant breeding, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Tiruvaipati Anusha
M.Sc student, Department of Genetics and Plant breeding, School of Agriculture
Lovely Professional University, Phagwara, Punjab, India

Dr. Shiv Prakash Shrivastav
Assistant Professor, Department of Genetics and Plant breeding
School of Agriculture
Lovely Professional University, Phagwara, Punjab, India

Corresponding Author
Kondeti Sai Sumanth
M.Sc Student, Department of Genetics and Plant breeding, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Transgenic crops: Present status, problems and future prospects

Kondeti Sai Sumanth, Tiruvaipati Anusha and Dr. Shiv Prakash Shrivastav

Abstract

Transgenic crops and genetic engineered plants have played critical roles in crop improvement by introducing advantageous foreign genes or inhibiting the expression of indigenous genes in crop plants. Transgenic crops are critical for biofortification, extending the shelf life of fruits and vegetables and generating pharmaceutical chemicals and therapeutic substances. With the expanding human population's need for food increasing, conventional methods of breeding enhanced output up to a certain point. However, new advancements in biotechnology such as transgenic crops and gene editing assist plants not only improve yield but also nutritional value. In at least one nation, 26 plant species have been genetically engineered and licenced for commercial distribution. In March 2002, the Government of India authorised Bt cotton as the first transgenic crop for commercial production for a three-year term. Aside from cotton, more than 20 crops are being researched and developed in India by over 50 public and private sector institutions. Thirteen of these crops have been allowed for restricted field testing in India. Potential growth is unlikely due to underinvestment in agricultural research, rising population pressure, and higher levels of resistance development in insect-pests to transgenic crops. However, broad acceptance of transgenic crops containing foreign genes is hampered by concerns about potential human toxicity and allergenicity, potential environmental dangers such as gene flow, deleterious effects on non-target animals, and resistance development in weeds and insects.

Keywords: Transgenic crops, GM crops, biofortification, gene gun, *Agrobacterium*-mediated transformation

Introduction

Transgenic crops are agricultural plants whose genomes have been altered using genetic engineering techniques to enhance existing traits or to bring in a new trait that does not occur naturally in the given crop species (Kumar *et al.*, 2020) [30]. During the 1970s, The Green Revolution in wheat and rice enabled India to become self-sufficient in food grain production. Climate change and rising population pressure have substantially altered the situation in the twenty-first century (Shukla *et al.*, 2018) [48]. Traditional technology will be unable to fulfil the food and nutrition needs of the future. When combined with conventional plant breeding approaches, advances in modern biology, particularly biotechnology and molecular biology, provide several benefits. Ti plasmid was proposed as a vector to introduce foreign genes into plant cells because of the natural ability of *Agrobacterium* tumefaciens to stably insert Ti plasmid DNA (T-DNA) into host plant cell genome was identified in 1977 (Chilton *et al.*, 1977) [7]. The first transgenic plants, antibiotic-resistant tobacco and petunia, were created in the same year (Fraley *et al.*, 1983; Herrera-Estrella *et al.*, 1983) [21, 25]. Approximately 525 distinct transgenic events in 32 crops have been authorized for production in various regions of the world to date. GM crop cultivation According to the most recent report, around 18 million farmers in 28 countries planted GM crops on 181.5 million hectares in 2014, representing a 3–4% growth over the previous year 2013 (Lucht *et al.*, 2015) [35]. The United States is the world's largest producer of GM crops, with around 73.1 million hectares under cultivation. Bt cotton, India's only GM crop, accounts for about (11.6 mha) 95 percent of total cotton producing area and ranks fourth in the world. Between 2002 and 2015, GEAC approved the commercial release of 1128 Bt cotton hybrid cultivars (Kumar *et al.*, 2018) [31]. The biggest producers and exporters of GM crops and goods are the United States of America, Argentina, and Canada (James 2010) China and India are the leading producers of transgenic crops in developing nations (James 2015). Tomato, corn, soybean, cotton, canola, rice, potato, squash, melon, and papaya are among the GM crops that have been commercialized in the last two decades, with soybean, corn, cotton, and canola being particularly important due to their widespread cultivation and importance in several countries' agricultural economies (Shukla *et al.*, 2018) [48]. Genetically engineered cotton (commonly known as Bt cotton) for insect resistance was approved for commercial cultivation in India in 2002 by the GEAC (Genetic Engineering Approval Committee), Government of India (GOI), and has proven to have become a major shift in Indian GM crop research, deregulation, and even for the cotton industry in India. Since then, Bt cotton has expanded at an exponential rate (Choudhary and Gaur 2010) [10]. Crop biofortification refers to the development of crops with increased nutritional value. This can be accomplished using either traditional selective breeding or genetic engineering. According to estimates, almost 800 million people worldwide are malnourished, with nearly 98 percent of them living in developing nations (Sinha *et al.*, 2019) [49]. Transgenic approaches for the creation of biofortified crops can be a viable option when there is insufficient genetic diversity in nutrient content among plant varieties (Brinch-Pedersen *et al.*, 2007) [5]. Nonetheless, advances in GM crops have sparked serious concerns about their safety and effectiveness. The GM seed business has been plagued by human health and pest resistance issues, which have substantially weakened its favorable impacts (Raman 2017) [44]. Some of the primary challenges for GM crop research and deregulation across the entire globe, including India, are the impact on the environment and ethical considerations. Some of the difficulties that need be addressed before releasing any GM crop for open field trials and commercialization are the ecological risk assessment of transgenic crops, the issue of gene flow, the development of secondary pest resistance, and the ecological hazards associated with pollen movement (Craig *et al.*, 2008) [14].

Development of transgenic crops

The most prevalent ways for incorporating foreign genes into plant cells are Agrobacterium-mediated transformation or direct gene transfer using a gene gun. The inherent capacity of soil bacteria of the genus Agrobacterium to behave as genetic engineers has been exploited. Since the mid-1990s, the introduction of super virulent strains of the bacterium has resulted in the effective development and recovery of transgenic plants in all major cereal crops and a variety of other crops that were previously transformable only by particle bombardment (Ishaida *et al.*, 1996) [26].

Agrobacterium-mediated transformation.

Agrobacterium's capacity to transfer genes into plant cells, where they are stably incorporated into the host chromosome(s) and expressed, has made these bacteria incredibly valuable in plant genetic engineering (Matthysse 2006) [38]. A tumor-inducing plasmid integrates the fragment of DNA that infects a plant into a plant chromosome (Ti plasmid). The Ti plasmid has the ability to manipulate the plant's cellular machinery and utilise it to replicate its own bacterial DNA. Ti plasmids are huge circular DNA particles that reproduce independently of bacterial chromosomes. This plasmid is essential because it contains transfer DNA (t DNA) areas where a researcher may introduce a gene that can be transmitted to a plant cell by a process known as the "floral dip" (Rani and Usha 2013) [45]. A Floral Dip is immersing flowering plants in an Agrobacterium solution containing the desired gene, followed by the collection of transgenic seeds straight from the plant (Chawla 2011) [7]. This practice is beneficial since it is a natural form of transmission and hence considered a more acceptable strategy. Furthermore, "Agrobacterium" is capable of effectively transmitting huge pieces of DNA. The integration of low copy number genes into plant chromosomes and the excellent quality and fertility of ensuing transgenic plants are the primary benefits of Agrobacterium-mediated gene transfer over physical approaches (Dai *et al.* 2001) [15].

One of the most significant disadvantages of Agrobacterium is that it cannot infect all key food crops. This approach is particularly effective for dicotyledonous plants such as potatoes, tomatoes, and tobacco plants (Chawla 2011) [7].

Gene gun method

Gene gun bombardment is a technique for physically introducing DNA into plant cells with cell walls. The gene cannon uses compressed helium as a propellant to bombard the plant cell wall with numerous DNA coated metal particles. Because the particles reach the cytosol and cell nucleus, the effectiveness of DNA transduction is far better than that of other techniques, requiring significantly lower dosages of DNA plasmid (Raska and milan 2015) [46]. Gold, tungsten, palladium, rhodium, platinum, and iridium are popular metal particles used in gene gun bombardment. They are DNA-coated, accelerated by helium gas, and then bombard the plant cells (Fang and Trewyn 2012) [20]. Using metal beads as carriers, gene gun integrates the DNA into the host genome. Particle bombardment has been widely used by plant biotechnologists since the early 1990s and has shown to be a highly successful strategy for gene transfer to plant cells (Altpeter *et al.*, 2005) [1]. The limitations of the gene gun approach include that particles might occasionally enter without carrying genetic material, causing cell harm, and that dna can sometimes get into unwanted cells, which is how herbicide resistance develops in weed plants (Demirer and Landry 2017) [17].

Advantages and disadvantages of transgenic crops.

Transgenic plants' key advantages include increased production, resistance to diseases and pests, and the ability to thrive under harsh conditions, while their main downsides include allergic responses, the rise of super-pests, and the loss of biodiversity. Herbicide resistance - Glyphosate is the active component in the majority of broad-spectrum herbicides. Glyphosate-resistant transgenic tomato, potato, tobacco, cotton, and other plants are created by re-assigning the aroA gene to a glyphosate EPSP synthetase from Salmonella typhimurium and E. coli. Insect resistance - During the last decade, transgenic cotton has significantly altered pest control in this crop. Cotton was one of the first Bacillus thuringiensis (Bt) insect-resistant and herbicide-tolerant (Ht) transgenic plants to be widely farmed. Growers can choose from over 300 transgenic cotton varieties expressing single or dual Bt proteins targeting lepidopteron larvae, as well as pyramided cultivars with herbicide resistance (Torres *et al.*, 2009) [52]. Medicinal use - Therapeutic proteins may be created in plants using recombinant DNA technology. Therapeutic proteins (used in the treatment and diagnosis of human illnesses) can be synthesised in plants. Molecular pharming is a field of study that combines agriculture with molecular biotechnology. Transgenic plants generate three forms of therapeutic proteins: (I) antibodies, (ii) proteins, and (iii) vaccines (Pusta *et al.*, 2008) [43]. Other advantages -

Genetically engineered food has improved its capacity to resist long-distance transportation. The GM crops were harvested while still green, allowing for ripening during transport and hence a longer shelf life. The product arrives at its destination without spoiling due to extended shipment and storage times. Improved nutritional value, use of marginalised land, and reduced environmental impact (Sayyeda and Singh 2021) [47].

According to the producers of these GM crops, adopting these seeds will provide various benefits, including improved yields and lower prices. In a world with billions of hungry mouths to feed, they pitch GM foods as a second "Green Revolution". Two major areas of worry have developed from the first generation of GM crops: environmental risk and human health risk. Transgenic crops have been linked to allergies in certain persons, while it is unclear if transgenic plants are to blame. There is a report on monarch butterfly larvae and other non-target species dying after swallowing transgenic maize pollens, the pollen of hybrid corn contains a bacterial poison.

Transgenic Crops for Biofortification

Crop biofortification refers to the development of crops with increased nutritional value. This can be accomplished using either traditional selective breeding or genetic engineering. Biofortification differs from fortification in that it tries to organically increase the nutritive value of plant foods rather than adding nutritional supplements to the foods during food processing (malik and maqbool 2020) [37]. The technique of creating a crop with bioavailable micronutrients in its edible components is known as biofortification. Such crops can be developed by either selective breeding or current biotechnology techniques (Garg *et al.*, 2018) [23]. According to estimates, almost 800 million people worldwide are malnourished, with nearly 98 percent of them living in poor nations (Sinha *et al.*, 2019) [49]. Biotechnological techniques, such as molecular marker-assisted section (Collard and Mackill, 2008; Moose and Mumm, 2008) [13, 39], are also used to help breeding operations, greatly increasing the success of breeding to improve crop nutritional value. Transgenic crops, such as golden rice, have genes inserted into their genomes to create micronutrients (Paine *et al.*, 2005) [41]. Because the intake and accumulation of micronutrients in edible sections of crops are regulated by polygenes with small effects, traditional breeding-based biofortification efforts have seen only limited success. (Naqvi 2009) [40]. Transgenics for nutrient biofortification should ideally meet two criteria: (1) selection of a broadly adapted genotype of an economically significant crop; (2) accumulation of nutrients in the edible section of the crop plant without compromising plant physiology or growth, and (3) economic yield (Vanderschuren *et al*. 2013) [54]. To increase the micronutrient content of cereal grains, several combinations of transgenic techniques have been developed and implemented. For example, iron biofortification in rice has been accomplished using various ways (Kok *et al*. 2018) [28].

Table 1: List of Biofortified crops developed using transgenes

Crop	Nutrient	Method	Gene Used	Reference
Rice	Vit A	*Agrobacterium*-mediated transformation	*PSY* and carotene desaturas	Ye *et al.*, 2000 [57]
	Folic acid (vitamin B9)	*Agrobacterium*-mediated transformation	Arabidopsis GTP-cyclohydrolase I	Storozhenko *et al.*, 2007 [50]
	Iron	*Agrobacterium*-mediated transformation	iron transporter *OsIRT1*	Takahashi *et al.*, 2001 [51]
		Agrobacterium-mediated transformation	nicotianamine synthase 1 (*OsNAS1)*	Lee and an 2009 [33]
		Agrobacterium-mediated transformation	nicotianamine aminotransferase	Zheng *et al.*, 2010 [59]
Wheat	provitamin A	Bombardment method	bacterial *PSY* and carotene desaturase	Wang *et al.*, 2014 [55]
	Iron	*Agrobacterium*-mediated transformation	ferritin gene	Xiaoyan *et al.*, 2012 [56]
Maize	provitamin A	*Agrobacterium*-mediated transformation	bacterial *crtB*	Aluru *et al.*, 2008 [2]
	Vit-E(Tocotrienol)	*Agrobacterium*-mediated transformation	homogentisic acid geranylgeranyl transferase HGGT	Cahoon *et al.*, 2003 [6]
	Vit-C	*Agrobacterium*-mediated transformation	dehydroascorbate reductase (DHAR)	Chen *et al.*, 2003 [8]
Sorghum	Vit-A	*Agrobacterium*-mediated transformation	*Homo188-A*	Lipkie *et al.*, 2013 [34]
	lysine	*Agrobacterium*-mediated transformation	high lysine protein	Zhao *et al.*, 2003 [58]
	Digestibility index	*Agrobacterium*-mediated transformation	RNAi silencing of the γ-kafirin	Elkonin *et al.*, 2016 [18]
Tomato	sterol	*Agrobacterium*-mediated transformation	3-hydroxymethl glutaryl CoA	Enfissi *et al.*, 2005 [19]
	phytoene	*Agrobacterium*-mediated transformation	1-deoxy-d-xylulose-5-phosphate synthase	Enfissi *et al.*, 2005 [19]
	lycopene, beta-carotene, and lutein	*Agrobacterium*-mediated transformation	*PSY* gene (*crtB)*	Fraser *et al.*, 2007
	carotenoid and flavonoid	RNA interference (RNAi) technology	photomorphogenesis regulatory gene DET1	Davuluri *et al.*, 2005 [16]
Alfalfa	methionine	electrophoresis	cystathionine γ-synthase (*AtCgS)*	Avraham *et al.*, 2005 [3]

Ethical concerns about transgenic crop

When transgenic plants are planted in the field, they will certainly come into touch with a variety of different species that work together to carry out a variety of ecological functions in agricultural areas. Despite the fact that there is now widespread scientific agreement that GE crops are safe to consume, many people remain apprehensive about this technology. The introduction of transgenic crops into the conventional food production system has caused uproar in society due to ethical and risk concerns. Five ethical issues have been expressed concerning GM crops: potential harm to human health, potential environmental damage, and detrimental influence on conventional agricultural practises, excessive corporate control, and the technology's 'unnaturalness'. The introduction of transgenic plants into the global situation, where food supply is struggling to keep up with an increasingly rising population, has sparked a firestorm of criticism (Choudhury *et al.* 2012) [11].

Human health - So yet, there is no definitive proof that genetically altered foods are more likely than traditional meals to trigger allergic responses. Genetic modification does not produce allergies. However, the type of the genes chosen for introduction into the modified host plant might cause allergic reactions. Horizontal gene transfer from genetically modified crops to intestinal microbiota is most likely caused by microbial transgenes (Kleter *et al.*, 2005) [27]. A transgenic plant has a portion of foreign DNA that is not found naturally in that plant. These DNA fragments are frequently derived from Trans (similar plants) or cis (completely distinct species), such as viruses and bacteria. There is a rising worry about concerns such as whether consuming "alien" DNA poses any risk to human or animal systems. (2013) (Bawa and Anilakumar 2013) [4]. According to (Lappe and bailey 1999), Roundup Ready soybeans had 12–14 percent less isoflavones than untreated soybeans. If Roundup Ready soybeans contain lower levels of isoflavones, they are less "good" for humans than regular soybeans. The negative environmental impacts of large-scale GM plant cultivation may have an indirect impact on human health. Concerns have been raised concerning GM plants' negative impact on the environment, animal populations, biodiversity, and gene transfer in non-GM wild herb species. Negative environmental impact of gm crops 1. The monarch butterfly 2. Crop-to-weed gene transfer 3. Resistance to antibiotics 4. GM protein leakage into soil 5. Pesticide spraying reductions: are they real? Crop hybridization with surrounding weeds may allow weeds to acquire features such as herbicide resistance that they do not normally have. According to research findings, crop traits can escape cultivation and survive in wild populations for many years. One specific risk associated with the planting of GM plants that has alarmed some people is gene flow from the modified plant to wild plants, particularly in regions of delicate biodiversity. More than 40 nations have passed legislation requiring the labelling of genetically modified foods (CBC News Online. 2004). Mandatory labelling legislation is urgently needed. However, the labelling is about more than simply health; it is also about consumer rights to make an educated decision regarding genetically modified food. Although GMF labelling is critical, it is doubtful that a universally agreed-upon labelling system will be established in the near future (Maghari and Ardekani 2011) [36]. Intellectual property rights (IPR) are a significant consideration in the present GMF discussion. Agri-business corporations patent GM crops, resulting in monopolisation of the global agricultural food supply and control over global food distribution. Social activists argue that the secret reason biotech businesses want to manufacture GM crops is because they can be privatised, as opposed to conventional crops, which are the inherent property of all people (Maghari and Ardekani 2011) [36]. The lengthy regulatory licencing procedure is a significant impediment to the quick adoption of transgenic crops. There is an urgent need for adequate science-based, cost-effective, and time-effective regulatory regimes that are accountable and rigorous but not burdensome for tiny and poor developing nations.

Future prospects of transgenic crops

One of the possible environmental benefits of transgenic plants (Kovalchuk *et al.*, 2001) [29] is its use in bioremediation, the process of decontaminating contaminated soils or water. The remediation potential of transgenic plants with the MerA gene from E. coli, which encodes mercuric ion reductase, is extremely useful. The possibility of GM crops as a production method for biodegradable polymers, notably poly (beta-hydroxybutyrate) (PHB) and poly (betahydroxyvalerate) (PBV), has been discussed (Poirier, 2001) [42]. Chymosin synthesis is a recent example of expression in transgenic plants of an animal enzyme utilized widely in the food business (Van Rooijen *et al.*, 2001) [53]. Although genetically modified crops have the potential to alleviate global food shortages, their practicality for production remains under doubt. The increased cultivation of GM crops to eradicate hunger has unintended consequences for the environment and human health. There many future uses of gm crops (i) improved and more efficient weed control; (ii) reduced losses due to insect pests and viruses and reduced need for insecticide; (iii) reduced post-harvest losses due to improved shelf life and marketing flexibility (tomato) due to storage pest resistance; (iv) increased nutritional quality (oil in canola); and (v) more effective hybrid seed production. Transgenic crops will be employed in the future not just for better agronomic qualities, but also for traits related to food processing, medicines (including edible vaccinations), and special chemicals.

Conclusion

Given the world's growing population, limited arable land area, and the rapid rate of climate change, there is an urgent need to produce high-yielding crop types that are nutritionally enhanced and resistant to a wide range of climates and biotic challenges. The benefits of transgenic insect-resistant crops have been demonstrated in terms of greater yields, fewer chemical inputs, and, as a result, enhanced farmer and consumer health. On the other hand, while there has been no indication of negative impacts, the possibility of pest resistance development and indirect damage to nontarget species warrants caution in how we deploy transgenic plants expressing insecticidal genes. Biofortification clearly has significant potential for increasing the nutritional content of important crops. The bioavailability of some critical minerals and vitamins might be enhanced by using recombinant DNA technology. One important source of concern, however, is that relatively few biofortified transgenic crops have been sold for mainstream cultivation. Even the well-known Golden Rice, which is biofortified with Vitamin A, was authorized by the FDA only after more than a decade of compliance. The practical use of transgenic crops may benefit developing nations' economies; thus, in my opinion, transgenic plants should be pushed in poor countries only after ensuring their non-harmful influence on natural resources and society. To prevent potential danger concerns, the influence of transgenic crops on ecosystems should be studied on a regular basis. Researchers expect to be able to give vaccines and medications through GM crops in the future, making it easier to provide medicine to people in developing countries. Food-based medications are easier to transport and keep than traditional medicines. Traditional technologies will be inadequate to meet the future's food and nutrition demands. Advances in modern biology, particularly biotechnology and molecular biology, give various advantages when paired with traditional plant breeding procedures. These are the only hope for future generations.

References

1. Altpeter F, Baisakh N, Beachy R, Bock R, Capell T, Christou P, *et al*. Particle bombardment and the genetic enhancement of crops: myths and realities. Molecular Breeding. 2005;15(3):305-327.

2. Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, Rodermel S. Generation of transgenic maize with enhanced provitamin A content. Journal of experimental Botany. 2008;59(13):3551-3562.
3. Avraham T, Badani H, Galili S, Amir R. Enhanced levels of methionine and cysteine in transgenic alfalfa (*Medicago sativa* L.) plants over-expressing the Arabidopsis cystathionine γ-synthase gene. Plant Biotechnology Journal. 2005;3(1):71-79.
4. Bawa AS, Anilakumar KR. Genetically modified foods: safety, risks and public concerns: A review. Journal of food science and technology. 2013;50(6):1035-1046.
5. Brinch-Pedersen H, Borg S, Tauris B, Holm PB. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. Journal of Cereal Science. 2007;46(3):308-326.
6. Cahoon EB, Hall SE, Ripp KG, Ganzke TS, Hitz WD, Coughlan SJ. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nature biotechnology. 2003;21(9):1082-1087.
7. Chawla H. Introduction to plant biotechnology. CRC Press, 2011.
8. Chen Z, Young TE, Ling J, Chang SC, Gallie DR. Increasing vitamin C content of plants through enhanced ascorbate recycling. Proceedings of the National Academy of Sciences. 2003;100(6):3525-3530.
9. Chilton MD, Drummond MH, Merlo DJ, Sciaky D, Montoya AL, Gordon MP, *et al*. Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of crown gall tumorigenesis. Cell. 1977;11(2):263-271.
10. Choudhary B, Gaur K. Bt cotton in India: A country profile. ISAAA Series of Biotech Crop Profiles. ISAAA: Ithaca, NY, 2010.
11. Choudhury AR, Das K, Ghosh S, Mukherjee RN, Banerjee R. Transgenic plants: benefits and controversies. J Bot. Soc. Bengal. 2012;66:29-35.
12. Clive J. 20th anniversary (1996 to 2015) of the global commercialization of biotech crops and biotech crop highlights in 2015. ISAAA Brief, 2015, (51).
13. Collard BC, Mackill DJ. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences. 2008;363(1491):557-572.
14. Craig W, Tepfer M, Degrassi G, Ripandelli D. An overview of general features of risk assessments of genetically modified crops. Euphytica. 2008;164(3):853-880.
15. Dai S, Zheng P, Marmey P, Zhang S, Tian W, Chen S, *et al*. Comparative analysis of transgenic rice plants obtained by Agrobacterium-mediated transformation and particle bombardment. Molecular Breeding. 2001;7(1):25-33.
16. Davuluri GR, Van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, *et al*. Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. Nature biotechnology. 2005;23(7):890-895.
17. Demirer GS, Landry MP. Delivering genes to plants. Chemical engineering progress. 2017;113(4):40-45.
18. Elkonin LA, Italianskaya JV, Domanina IV, Selivanov NY, Rakitin AL, Ravin NV. Transgenic sorghum with improved digestibility of storage proteins obtained by Agrobacterium-mediated transformation. Russian Journal of Plant Physiology. 2016;63(5):678-689.
19. Enfissi EM, Fraser PD, Lois LM, Boronat A, Schuch W, Bramley PM. Metabolic engineering of the mevalonate and non-mevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. Plant Biotechnology Journal. 2005;3(1):17-27.
20. Fang IJ, Trewyn BG. Application of mesoporous silica nanoparticles in intracellular delivery of molecules and proteins. Methods in Enzymology. 2012;508:41-59.
21. Fraley RT, Rogers SG, Horsch RB, Sanders PR, Flick JS, Adams SP, *et al*. Expression of bacterial genes in plant cells. Proceedings of the National Academy of Sciences. 1983;80(15):4803-4807.
22. Fraser PD, Enfissi EM, Halket JM, Truesdale MR, Yu D, Gerrish C, *et al*. Manipulation of phytoene levels in tomato fruit: effects on isoprenoids, plastids, and intermediary metabolism. The Plant Cell. 2007;19(10):3194-3211.
23. Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, *et al*. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Frontiers in Nutrition, 2018, 12.
24. Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, *et al*. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Frontiers in Nutrition, 2018, 12.
25. Herrera-Estrella L, Depicker A, Van Montagu M, Schell J. Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. Nature. 1983;303(5914):209-213.
26. Ishida Y, Saito H, Ohta S, Hiei Y, Komari T, Kumashiro T. High efficiency transformation of maize (*Zea mays* L.) mediated by Agrobacterium tumefaciens. Nature biotechnology. 1996;14(6):745-750.
27. Kleter GA, Peijnenburg AA, Aarts HJ. Health considerations regarding horizontal transfer of microbial transgenes present in genetically modified crops. Journal of Biomedicine and Biotechnology. 2005;(4):326-352.
28. Kok ADX, Yoon LL, Sekeli R, Yeong WC, Yusof ZNB, Song LK. Iron biofortification of rice: Progress and prospects. F. Shah, ZH Khan and A. Iqbal (Norderstedt: IntechOpen, 2018, 25-44.
29. Kovalchuk I, Kovalchuk O, Hohn B. Biomonitoring the genotoxicity of environmental factors with transgenic plants. Trends in Plant Science. 2001;6(7):306-310.
30. Kumar K, Gambhir G, Dass A, Tripathi AK, Singh A, Jha AK, *et al*. Genetically modified crops: current status and future prospects. Planta. 2020;251(4):1-27.
31. Kumar M, Singh SP, Kumar M, Kumar A, Kumar S, Kumari P. Biosafety issues in commercialization and development of transgenic crops. Int. J Curr. Microbiol. App. Sci. 2018;7(4):2161-2174.
32. Lappe M, Bailey B. Against the grain: biotechnology and the corporate takeover of your food, 1998.
33. Lee S, An G. Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. Plant, Cell & Environment. 2009;32(4):408-416.
34. Lipkie TE, De Moura FF, Zhao ZY, Albertsen MC, Che P, Glassman K, *et al*. Bioaccessibility of carotenoids from transgenic provitamin A biofortified sorghum. Journal of agricultural and food chemistry.

2013;61(24):5764-5771.
35. Lucht JM. Public acceptance of plant biotechnology and GM crops. Viruses. 2015;7(8):4254-4281.
36. Maghari BM, Ardekani AM. Genetically modified foods and social concerns. Avicenna journal of medical biotechnology. 2011;3(3):109.
37. Malik KA, Maqbool A. Transgenic crops for biofortification. Frontiers in Sustainable Food Systems. 2020;4:571402.
38. Matthysse AG. The genus agrobacterium. Prokaryotes. 2006;5:91-114.
39. Moose SP, Mumm RH. Molecular plant breeding as the foundation for 21st century crop improvement. Plant physiology. 2008;147(3):969-977.
40. Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, *et al*. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proceedings of the National Academy of Sciences. 2009;106(19):7762-7767.
41. Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, *et al*. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nature biotechnology. 2005;23(4):482-487.
42. Poirier Y. Production of polyesters in transgenic plants. Biopolyesters, 2001, 209-240.
43. Pusta D, PAŞCA I, Morar R, Sobolu R, RĂDUCU C, Odagiu A. The transgenic plants–advantages regarding their cultivation and potentially risks concerning the food safety. Journal of Central European Agriculture. 2008;9(4):785-788.
44. Raman R. The impact of Genetically Modified (GM) crops in modern agriculture: A review. GM crops & food. 2017;8(4):195-208.
45. Rani SJ, Usha R. Transgenic plants: Types, benefits, public concerns and future. Journal of Pharmacy Research. 2013;6(8):879-883.
46. Raska M, Turanek J. DNA vaccines for the induction of immune responses in mucosal tissues. In Mucosal immunology. Academic Press, 2015, pp. 1307-1335.
47. Sayyeda S, Singh VP. Transgenic Plants: Advantages and Disadvantages. Biotica Research Today. 2021;3(9):721-724.
48. Shukla M, Al-Busaidi KT, Trivedi M, Tiwari RK. Status of research, regulations and challenges for genetically modified crops in India. GM crops & food. 2018;9(4):173-188.
49. Sinha P, Davis J, Saag L, Wanke C, Salgame P, Mesick J, *et al*. Undernutrition and tuberculosis: Public health implications. The Journal of infectious diseases. 2019;219(9):1356-1363.
50. Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, *et al*. Folate fortification of rice by metabolic engineering. Nature biotechnology. 2007;25(11):1277-1279.
51. Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S. Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. Nature biotechnology. 2001;19(5):466-469.
52. Torres JB, Ruberson JR, Whitehouse M. Transgenic cotton for sustainable pest management: A review. Organic farming, pest control and remediation of soil pollutants, 2009, 15-53.
53. Van Rooijen G, Keon RG, Boothe J, Shen Y. Commercial production of chymosin in plants. Patent Application WO 01/14571, 2001.
54. Vanderschuren H, Boycheva S, Li KT, Szydlowski N, Gruissem W, Fitzpatrick TB. Strategies for vitamin B6 biofortification of plants: a dual role as a micronutrient and a stress protectant. Frontiers in plant science. 2013;4:143.
55. Wang C, Zeng J, Li Y, Hu W, Chen L, Miao Y, *et al*. Enrichment of provitamin A content in wheat (*Triticum aestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes CrtB and CrtI. J Exp Bot. 2014;65(9):2545-56. doi:10.1093/jxb/eru138.
56. Xiaoyan S, Yan Z, Shubin W. Improvement Fe content of wheat (*Triticum aestivum*) grain by soybean ferritin expression cassette without vector backbone sequence. Journal of Agricultural Biotechnology, 2012.
57. Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, *et al*. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science. 2000;287(5451):303-305.
58. Zhao ZY, Glassman K, Sewalt V, Wang N, Miller M, Chang S, *et al*. Nutritionally improved transgenic sorghum. In Plant biotechnology 2002 and beyond Springer. Dordrecht. 2003;413-41(6).
59. Zheng L, Cheng Z, Ai C, Jiang X, Bei X, Zheng Y, *et al*. Nicotianamine, a novel enhancer of rice iron bioavailability to humans. PLoS One, 2010;5(4):e10190.