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Genetically Modified Crops

Current Status, Prospects and Challenges Volume 1

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Foreword

To get easy access to food and to improve the productivity of crop plants, humans have used methods of domestication and improvement through selective breeding, based on useful phenotypic traits. It was through the work of Gregor Mendel that we learnt about the genetic basis of plant traits. The first hybrid corn was developed in 1922 by an intelligent breeding strategy. Following the discovery of DNA as the genetic material, the work of a number of groups led to the concept of gene as the unit of DNA that controls a phenotypic character of an organism. And it was in 1973 that Herbert Boyer and Stanley Cohen developed genetic engineering by inserting DNA from one bacterium to another. Around the same time Jeff Schell and Marc Van Montagu discovered that it is due to the transfer of the plasmid DNA of *Agrobacterium tumefaciens* that results in tumor formation in plants. This research was a by-product of curiosity-driven science and based on fundamental scientific discovery. Using this information and developing plant transformation technology, the group of Mary-Dell Chilton and R. Fraley and scientists from Monsanto Company created the first transgenic plant. During the mid-1990s, with the creation of GM tomato, the initial wave of GM plants was set in motion. However, due to certain issues of public acceptability and stringent regulatory laws that were put in place in different countries, the growth of this technology was slowed down. Van Montagu, whom I have had the pleasure of meeting and knowing for a long time, wrote an insightful article in the *Annual Review of Plant Biology* in 2011 titled, “It is a long way to GM agriculture.” Even then this technology has been used in many crops, and the global biotech crop area is steadily increasing within many countries which have adopted this technology for crop improvement in their agriculture systems. Unfortunately, due to various social and political issues the adoption of this technology has received resistance. This trend needs to be reversed. In the meanwhile, one has seen the emergence of new technologies like RNAi to silence the expression of genes to understand their role as also to develop novel transgenic plants with useful traits. And since 2015, gene editing technologies have evolved which have become useful and efficient tools to manipulate DNA in plant cells. And now we are moving onwards to precision genome engineering through prime genome editing, which does not involve double-strand breaks and donor DNA templates. Hopefully, these interventions will not be subjected to as much stringent regulatory procedures and will also find better acceptability in the society.

An article was published in *EMBO Reports* by Fagerstrom et al. in 2013, entitled "Stop Worrying Start Growing" with the subtitle, "Risk research on GM crops is a dead parrot: it is time to start reaping the benefits of GM." This is even more true today. The present volumes by Professors Kavi Kishor, Rajam, and Pullaiah have been compiled to convey the same message by presenting achievements and opportunity of employing different technological tools for genetic improvement of plants. I have known the editors of this volume for a long time. They have themselves made significant contributions in the area of plant biotechnology and are well acquainted with GMOs, in all its perspectives. They are also aware of the views of opponents of this technology. Accordingly, taking these into considerations, they have broadly outlined the status, prospects, and challenges of different genetic interventions in various plants of economic importance for improving traits like developing resistance to viral, insect, and other diseases and for conferring tolerance to abiotic stresses. With rapid advancements in genome sequencing methodologies and functional genomics tools, it has now been possible to identify the genes that can be deployed in a very precise manner using efficient transformation techniques.

These volumes cover, among cereals, a chapter on rice that deals with the use of GM technology to address the problem of food and nutrition security and a chapter each on wheat and finger millet. Legumes, which remained recalcitrant for a long time and an efficient transformation system was not available, have now been tamed. This family of plants have received special attention, and a chapter each on pigeonpea, chickpea, cowpea, and peanut has found a place in this volume. Among vegetables there is a detailed account on the present status on brinjal, tomato, and cucurbits and one chapter each on redpepper and capsicum. Other plants of importance which have been included are sugarcane, cassava, banana, papaya, citrus, mulberry, and jatropha. The work on two oil plants, sunflower and safflower, has been presented in two independent chapters. This approach of illustrating the use of the technology for each species separately, rather than group them on specific trait, I find, provides a better perspective to evaluate the importance of GM technology with respect to each plant species.

These volumes, I am very sure, will be useful to all students and practitioners of biotechnology, be in colleges, universities, and private organizations, as well as for policy makers and regulators in the government agencies. I look forward to the deployment of the safe use of new tools and techniques of genetic manipulation for the improvement of important plants on a large scale in our agriculture and horticulture system. This will help, along with other breeding methodologies, including marker-assisted breeding, to sustain productivity with limited inputs. We hope to see a hunger-free world in the years to come.

International Centre for Genetic Engineering
and Biotechnology, New Delhi, India
June 06, 2020

Sudhir K. Sopory

Preface

Plants provide us many essential things in life, including food, feed, cloth, wood, paper, medicinal compounds, industrial products, and most importantly the life-sustaining molecule oxygen to breath. Plants are also crucial to clean lifesaving water. There are only six crop plants, *viz.*, rice, wheat, corn, potato, sweet potato, and cassava, which provide about 80% calories to humans. There are other important crops like sugarcane, barley, sorghum, bean, soybean, coconut, and banana, which are also being consumed by humans. But crop plants are vulnerable to various biotic factors (pathogens and pests) and extreme environmental conditions or abiotic stresses (e.g., high salinity, drought, heat and cold, heavy metals, and submergence) because of their sessile nature. These stresses cause a colossal loss of crop yields and impair nutritional quality. Otherwise, one can realize the potential and harvest 100% agricultural productivity from all crops. In addition, global warming, shrinking water resources, arable land, and population growth are aggravating the problem of food security. In fact, these are key scientific issues in agriculture besides post-harvest losses and impairment in nutritional quality. Then the critical question that arises in our minds is how to harness the full yield potentials of crops without compromising the quality component. The answer lies evidently in the exploitation of diverse technologies, particularly plant breeding and genetic engineering. Between plant breeding and genetic engineering, the former has contributed significantly for more than seven decades to crop improvement and in fact almost all the new and improved varieties were virtually derived through breeding strategies. However, breeding methods suffer from certain limitations like incompatibility barriers or narrow mobilization of useful genes between closely related species. This leads to the problem of using only limited gene pool and there is no way to transfer a single beneficial gene since we generally transfer a cluster of genes/chromosomes during the crosses, thus subjecting F_1 hybrids for 4–5 backcrosses to chuck away the unacceptable. It takes nearly 10–12 years to develop a new variety with desirable traits and may not be cost-effective. In contrast, genetic transformation by *Agrobacterium* or other gene delivery systems or transgenic technology offers several advantages such as precise gene transfer from any source to crop plants. This means a huge gene pool exists for the transfer of desirable traits across species and takes relatively 7–9 years to develop a transgenic line of interest. Consequently, genetic engineering holds great promise for crop improvement and is essential since huge gap exists between food production and rate of population growth. Today's world population is about 7.7 billion and is expected to reach 9.7 billion by 2050, and further to an estimated 11 billion by 2100. Human hunger and malnutrition are the major problems, especially in Asian countries due to accelerating birth rates. So, it is a challenge for plant biologists and biotechnologists to resolve the problem of human hunger and malnutrition through crop improvement programs. In reality, about 70% increase in food production is required by 2050 to feed the growing masses; otherwise we may face great famines in the near future. Indeed, this suggests that a second green revolution is the need of the hour to bring food security to the world population, and this can only happen if we couple the conventional breeding strategies with genetic engineering technologies.

Transgenic technology has already proven to be novel and a potential alternative for crop improvement, and a handful of transgenic varieties like cotton, corn, soybean, and canola have been commercialized globally. This has led to a substantial increase in crop yield and quality, reduced use of harmful pesticides, reduction in CO_2 emissions, and decrease in the cost of crop production, besides improving the economy of marginal farmers. The first transgenic variety, *flavr savr*—the slow ripening tomato, was commercialized in 1994 in the USA, and since then there is a steady increase in the adoption of the first generation of genetically modified (GM) crops such as corn, cotton, and soybean for insect resistance, herbicide tolerance, and improvement of oil quality. In 2018, about 475 million acres (191.7 million hectares) of land was under the cultivation of various GM crops in 26 countries (21 developing and 5 developed countries), including 5 top countries—USA, Argentina, Brazil, Canada, and India (with the adoption of only Bt cotton) with the largest area of GM crops grown, and an additional 44 countries imported these GM crops. To date, about 525 different transgenic events in 32 crops have been approved for cultivation in different parts of the world. Currently, the next generation of transgenic plants displayed potential for the production of bio-ethanol, bio-plastics, and many pharmaceutically important recombinant proteins and compounds. Interestingly, the recent genome engineering or editing technology is quickly gaining importance for maneuvering genes in crop plants using the gene editing tool, the CRISPR-Cas system. This technology is aiding us in the improvement of many agronomically important traits such as yield, stress tolerance, and nutritional quality. Soon, the gene-edited crop plants with new traits, but not having an alien gene, will be commercialized. Such an endeavor will assist us in meeting the increasing food demands and global food security. This technology can be safely exploited since it has minimum or no regulatory issues. GM crops have the most rapid adoption rate in the history in spite of public concerns as compared to the traditional hybrids like corn, which took more than seven decades for global penetration. Transgenic varieties were released only after passing the tests against environmental aggressiveness, toxicity, allergenicity, after fulfilling the stringent regulatory guidelines laid down by the respective countries, and after exhibiting their superiority for field performance vis-à-vis the untransformed or wild-type plants.

The present book brought in two volumes has updated information about the current status of GM crops. While the first volume covered genetic modification studies in cereals, pulses, and oil-yielding crops, the second one included information on important vegetable, fruit-yielding, and commercial crops. These volumes on GM crops will be handy to students of life science stream of both undergraduate and postgraduate studies, research scholars, postdocs and researchers working in plant and agricultural biotechnology organizations, faculty members, biotech companies, and professionals alike.

Lastly, we would like to express our heartfelt gratitude to Springer-Singapore for kindly consenting to bring out this book in two volumes and for extending support through various phases and for the timely completion of publishing. Our heartfelt thanks are also due to Prof. Sudhir K. Sopory, ICGEB, New Delhi, for writing the foreword. We would like to thank all the authors/coauthors who have contributed the review articles and also for their cooperation and erudition.

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T. Pullaiah is a former Professor in the Department of Botany at Sri Krishnadevaraya University in Andhra Pradesh, India. He has held several positions at the university and was President of the Indian Botanical Society and of the Indian Association for Angiosperm Taxonomy. He holds a PhD from Andhra University, India, and was a Postdoctoral Fellow at Moscow State University, Russia.

He was awarded the Panchanan Maheshwari Gold Medal, the Dr. G. Panigrahi Memorial Lecture award of the Indian Botanical Society, and the Prof. Y.D. Tyagi Gold Medal of the Indian Association for Angiosperm Taxonomy. He has authored 51 books, edited 19 books, and published over 330 research papers. He was a member of the Species Survival Commission of the International Union for Conservation of Nature (IUCN).

Genetic Tinkering of Crops for Sustainable Development: 2020 and Beyond

P. B. Kavi Kishor, M. V. Rajam, and T. Pullaiah

Abstract

The advent of gene isolation from diverse organisms and their transfer into different vectors along with promoters and selectable marker genes are the milestone events in the annals of molecular biology. Further, varied efficient protocols developed for transferring alien genes into the host genomes have unfolded the evolution of transgenic plants for biotic and abiotic stress tolerance, nutritional quality improvement and refinement of many other agronomically important traits. Such transgenic events if occupy the agricultural landscape world over can not only aid to meet the evergrowing food demands alongside the nutritional quality but also help us in sustainable development. Ongoing endeavours all over the world in different laboratories showcased the development of genetically modified (GM) crop plants using candidate genes with different promoters. This has proved beyond doubt that the genetic engineering technologies evolved over time are robust and reproducible. Though a large number of candidate genes including transcription factors have been transferred for conferring diverse agronomic traits, majority of them have not been tested in the open fields and not released for the consumption of general public. Governments across the globe are exercising a caution with the apprehension of spread of engineered genes into the wild species and environmental degradation too. Effective measures and policies therefore must be evolved to clear the uncertainties/anxieties raised by the

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general public and environmentalists alike for the safety of our environment before the release of transgenic crop plants into the open fields.

Keywords

Transgenic plants · Genetic modification · Stress tolerance · Nutritional improvement · Sustainable development · Biosafety

1 Introduction

There is a huge demand for food as the world's human population is expected to reach 9.7 billion by 2050 and further to an estimated 11 billion by 2100 (Raman 2017). Moreover, there are many challenges in agriculture, including the shrinking of resources like water and arable land for crop production, crop yield loss due to pathogens and pests, post-harvest losses, etc. Therefore, the enhancement of food production by both conventional and non-conventional approaches is a matter of the utmost importance to bridge the gap between population growth and food production, and food security, if not taken care, might lead to great famines in the foreseeable future. In this regard, the transgenic technology appears to be a novel and potential alternative to enhance the food production, achieve food security and alleviate the human hunger and malnutrition. In fact, the biotechnological intervention, particularly genetically modified (GM) crops has been proposed to lessen the environmental footprint by improving food quality and enhancing crop productivity (Barros et al. 2019).

Deliberate manipulation of the genes using diverse methods of gene transfer generates transgenic or GM crops (Hundleby and Harwood 2019). Many countries are now able to grow transgenics that help farmers to significantly enhance crop productivity by ~22%, reduce the dependency on agro-chemicals (pesticides) by ~37% for controlling against various biotic stresses and also increase farmer profits by ~68% (Klümper and Qaim 2014; Gruissem 2015). In the USA, transgenic corn acreage is seeded with 92% of the GM crop growing area in 2018 compared with 85% in 2009 and 25% in 2000 (NRC 2002, 2018). There is an overall agreement that our agricultural landscape covering transgenics has improved the yields world over in varied crops showcasing the evidence what genetic engineering technology can do. Despite uncertainties in the field to accept GM crops by the consumers, the potential of the technology is enormous as evident from the experimental material being tested across many countries over a period of time. This introductory chapter focuses on what transgenic lines are being grown or under sale for use with desirable traits alongside their benefits in the wake of climate change.

2 GM Crops Currently Being Grown

The first generation of transgenic crops was raised based on single-gene transfers. Flavr Savr tomato was the first GM crop developed using a single gene and introduced in the USA in the year 1994 (Kramer and Redenbaugh 1994). Flavr Savr tomato has been modified genetically to slow down the process of fruit ripening, cell wall softening and rotting. Though gene transfer technology using *Agrobacterium*-mediated gene transfer into tomato was robust and reproducible, the Flavr Savr tomato produced was not successful as a commercial crop. In 1996, 1.7 MHa of GM crops were planted all over the world, but by 2015, the GM crop-growing area was increased to 179.7 MHa. Over 10% of the world's land (179.7 million hectares) was used to grow GM crops in 28 countries by the year 2015, and the acreage is increasing year after year. While the USA grows nearly 71 million hectares (MHa), smaller countries like Argentina (24.5 MHa) and Brazil (44.2 MHa) also grow GM crops in huge amounts of their agricultural areas. India grows only GM cotton in approximately 11.6 MHa (Dunwell 1998, 1999; Raman 2017). Besides, controlled trials are still being tested in several countries including the UK, Africa and Canada. Major crops being grown commercially include aubergine or brinjal (Bangladesh), cotton (nearly 15 countries), maize (17 countries), oilseed rape (Canola) (4 countries including Canada), papaya (the USA, China), potato (the USA), soybean (11 countries), squash (the USA) and sugar beet (North America). While GM crops such as soybean accounts for 83% of the world production (92.1 MHa), cotton represents 75% in the year 2015 (Raman 2017). Several of the European countries like Spain, Portugal, Romania and Slovakia grow mostly maize, but not other crops. Many GM crops produced in the mid-1990s protected the crops against pathogens, insects and herbicides. Though crop plants with abiotic stress tolerance were developed, they were not tested at the field level barring corn (Dunwell 1999; Checker et al. 2012; Chang et al. 2014). Transgenic drought tolerant corn was developed but not yet released to the farmers. Some of the transgenic crops like soybean which is glyphosate resistant, cotton and corn resistant to insects due to *Bt* genes attained commercial success (Dunwell 1996, 1999; James 1998, 2011). Thus, the first-generation transgenics included several crops that were resistant mostly to biotic stresses (Raman 2017; Askari-Khorasgani and Pessarakli 2018).

In 2015, while the USA grew ten GM crops, Canada produced only four varieties. GM varieties like alfalfa, apple, eggplant, poplar, potato and squash were grown in one country each. In 2015, Brazil had approved GM crops like *Phaseolus vulgaris* and eucalyptus for commercialization. Likewise, transgenic rice, wheat, sorghum, cassava, banana, camelina, citrus, chickpea, cowpea, groundnut, mustard, pigeon pea, chestnut (*Castanea dentata*) and safflower (*Carthamus tinctorius*) were in various stages of progress (James 2014, 2015). Data for commercially grown GM varieties are available for nine food crops, three non-food crop plants and also two types of flowering plants for the year 2015 (James 2015). Among them, maize and soybean crops were the widely grown across the globe. In 2018, a total of 70 countries adopted GM crops through cultivation and importation (NRC 2018). About 191.7 million hectares of GM crops were planted in 26 countries (21 developed and

5 industrialized). The USA, Brazil, Argentina, Canada and India are the top five countries with the largest area of GM crops planted, collectively occupying 91% of the global GM crops area. The cultivation of new-generation herbicide tolerant cotton and soybean, low gossypol cotton, roundup ready (RR) and low lignin alfalfa, omega-3 canola and insect resistant (IR) cowpea has been approved for plantation in 2019 (NRC 2018).

3 Commercial GM Crops and Vandalism

Regrettably, general public is still not acceding and endorsing the GM food. Destruction of public and governmental experiments of GM crops were reported in many countries during trials in the open fields. Kuntz (2012) reported destruction of a trial of a wide variety of GM crops in France, Germany, the UK and Switzerland. The loss from such damage has been estimated at 1.2 million Euros. Sadly, there is a widespread rejection of GM foods all over the world. This is something worth pondering in the right spirit, understood and debated from different sections in the scientific circles, politicians, policymakers, nongovernmental organizations and general public. GM crops draw the public attention and hence needs discussions. Needless perhaps to mention that genetic engineering is not discovered by humans, but a naturally happening phenomenon. It is a continuous process. The fact is that every organism is genetically modified, but naturally. Utilization of GM crops must be discussed and debated in this context for the larger benefits of the society. GM crops have been rejected by European Union (EU), yet large number of European countries import GM agricultural products like soybean meal and soybeans as a feed for livestock. European countries import GM soybean meal and soybean from Argentina, Brazil and the USA to the tune of $9 and $6.5 billion per year, respectively (Dunwell 2014). How can countries that do not grow GM crops in their own farm lands are importing from other countries? (Masip et al. 2013). That is seemingly absurd and certainly paradoxical.

4 The Second-Generation Transgenics with Industrial Applications

While the first-generation transgenics were concentrated on transfer of single genes that influenced distinct agronomic characters, researchers then focussed to develop transgenics with a wide spectrum of genes that influenced industrially important products. Such a product generation would depend upon genome-wide screening, identification and validation of candidate genes. The appearance of next-generation transgenics greatly impacted the environment and industry. Transgenic yellow poplar (*Liriodendron tulipifera*) with bacterial mercuric reductase gene can now be used for phytoremediation of industrial wastes such as ionic mercury (Rugh et al. 1998). Transgenics release elemental mercury at significantly higher levels compared to wild-type plants. Transgenic mustard with increased tolerance to cadmium

(Zhu et al. 1999) and tobacco engineered for degrading hydrocarbon pollutants have also been generated (Dunwell 1999).

4.1 Bioenergy and Ethanol Production

It is known that poplar and eucalyptus are being used as feedstock for the production of ethanol. By improving cellulose content using biosynthetic pathway gene manipulations, Arioli et al. (1998) and Hu et al. (2013) generated transgenics with improved biomass. Similarly, overexpression of cellulase enzyme resulted in improved ethanol production to be used in automobile industry (Lebel et al. 1998). Further, reduction in lignin by downregulation of lignin biosynthetic pathway genes improved cellulosic biomass and alcohol production (Bauscher et al. 1998; Lapierre et al. 1999; Prashant et al. 2011; Hu et al. 2013).

4.2 Bioplastic Industry

Since plastics have been banned, bioplastics need to be developed. Poirier (1999) has reported that Monsanto developed a transgenic oilseed rape which expresses polyhydroxybutyrates (PHB) in the leucoplasts nearly 8% of its dry biomass. As a novel concept, the genes have been expressed in rubber trees. This helps us to harvest the bioplastics continuously by tapping the latex and without demolishing the plants (Arokiaraj et al. 1998).

4.3 Coloured Cotton and Textile Industry

Cotton is best known for its insect resistance with the incorporation of *Bt* gene. It is being grown widely in several countries including India (John 1997; Dunwell 1999). But introduction of pigment compounds such as melanin for black colouration and also other colours would be of interest since it can preclude the dyeing of cotton fabric. Permission to grow such transgenic coloured cotton would help the textile industry. Use of fibre-specific promoters can help create such fabrics which is certainly the need of the hour. Natural brown and green-coloured fibres exist but poor fibre quality limits the utility of such coloured cotton (Liu et al. 2018). Therefore, transgenic coloured fibres were developed which is of immense help to the mankind. But like natural coloured fibres, transgenic coloured fibres are not only weaker but also shorter than wild-type controls (Liu et al. 2018). Thus, it is clear that potential exists for the genetic manipulation of flavonoid biosynthetic pathway genes to alter the colour of cotton fibre as well as quality. Further, it is of interest to note the synthesis of polyhydroxybutyrates in the fibre cells has helped thermal properties of the cotton fibre (Chowdhury and John 1998; Hankermeyer and Tjeerdema 1999; Poirier 1999). Thus, the textile industry would be benefitted if the GM plants are permitted to grow.

4.4 Paper and Pulp Industry

Reduction in lignin content has a bearing in pulping process and paper industry. Field trials of several transgenics are still on and pulping tests are being conducted for use of GM plants in paper industry (Bauscher et al. 1998). If such transgenics are brought to use, the paper industry would be massively benefitted.

4.5 Production of Terpenoids and Mint Oil

Several mono- and sesquiterpenes are used in flavour, perfume and pharmaceutical industries. One such molecule is mint oil with nearly $6 billion industry including its processed products (Lange and Croteau 1999). Genetic manipulation for the production of mint oil, especially *p*-menthane monoterpene metabolism in peppermint industry has resulted in (−)-menthone to (−)-menthol (Lange and Croteau 1999). Likewise, attempts to increase the density of glandular trichomes of *Mentha* species are being made. If they succeed, GM crops with better yields of terpenoid compounds would be available for use in flavour and fragrance industries.

4.6 Transgenic Plants in Pharmaceutical Industry and Veterinary Applications

Transgenic plants have been developed for the production of many pharmaceutically important compounds, valuable chemicals, vaccines, antigens, antibodies, enzymes and growth factors (Lee et al. 1997; Arakawa et al. 1998; Gruber et al. 1998a; Somerville and Bonetta 2001; Daniell et al. 2001; Fischer et al. 2004; Ortiz and Swennen 2014; Ankita et al. 2016). Among plant-derived compounds under the category, plant protein toxin called ricin produced by the *Ricinus communis* has considerable use in pharmaceutical industry as a therapeutic agent (in cancer and apoptosis). Sehnke and Ferl (1999) produced safe recombinant ricin, but not yet commercialized. More importantly, human haemoglobin (Dieryck et al. 1997) and collagen (Gruber et al. 1998b) have been produced in plants. These products have the potential for commercialization but have not been launched. Environmental effects of transgenic plants have been thoroughly discussed at diverse fora by scientific experts (Domingo 2016; Kumar et al. 2018; Giraldo et al. 2019), but the scope and adequacy of regulation is always under hammer in majority of the countries (National Research Council 2002). Domingo (2016) reported that the assessed GM soybeans, rice, maize and wheat are shown to be safe like that of parental species. Where controversies exist, there he noticed lack of proper reports for many GM crops. The report of WHO as well as the assessment of published literature by Domingo (2007) reveals that the GM products (canola, corn, cucumber, peas, pepper, potatoes, rice, sweet pepper, soybean, and tomatoes) being used currently on the international market have passed risk assessments conducted by respective national authorities. Not surprisingly, different assessments have not recorded any potential toxicity of GM or risk to human health (Domingo 2007). Gene flow has occurred from transgenics to wild/related species, but no one cited any example that demonstrated an adverse environmental effect of such a gene flow from GM crops. However, long-term studies are crucial on the safety and health effects of GM crops with reliable scientific data. The National Research Council recommends "public-sector investment in GM crop risk analysis, better methodologies and protocols for development of GM plants". Committee on GM crops assessed the rigour of all available evidences that support or negate the claims about the potential human health risks/benefits of several GM foods (NASEM 2016). Further, FDA in the USA have not allowed any GM food until such food is proven safe for human consumption (NASEM 2016). The outcry by the researchers for the legitimate release of GM crops is valid, but it is perhaps vital to improve the transgenic methods that will reduce the risks to the environmental safety. It is also recommended that GM crops must be subjected to safety testing if they have intended or unintended qualities if any with potential hazards to animals and humans. A comparison of the molecular profiles of the GM crops with those of their counterparts already in use is perhaps recommended. Also, the governance of all GM crops should be transparent and participatory before they are released to the public. This would instil confidence and also widespread acceptance among the consumers. It is therefore essential to monitor GM crops for the effects on the environment, the spread of transgene to the wild relatives, on animal and human health and also intense research on social, economic and value-based issues that damage and devastate our precious environment. Research on such aspects is urgently warranted since we need to bring the fruits of GM crops on to the table by minimizing the environmental and human health risks if any.

5 Policy Issues

- Policy issues may change as the type of transgenics changes. But, research funding for hazard identification and risk assessment studies is meagre world over.
- We need to develop scientifically sound protocols to find out if the transgenes are causing any damage to the environment (Devos et al. 2016) and also to the non-target organisms. Protocols available at our disposal today are effective in finding out the toxic chemicals being spread if any and the sequence of their broad ecological consequences.
- The effect of horizontal transgene transfer to pollinators, soil microbiota and conservation of species must be evaluated for several seasons across the countries (Giacomo et al. 2016).
- Further, the movement of transgene if any needs to be traced in the wild relatives.
- It is also vital for us to comprehend if the genetic modifications are affecting the invasiveness of the species.
- Regulatory systems that are in place across the globe must be effective and efficient to assess the GM crops and the ecological damage, animal and human health risks if they are causing. Existing regulatory issues need to be strength-

ened, improved and modified. Such regulatory policies must be based on our vast experience, sound scientific principles and methodologies being used.

- Possible environmental hazards or ecological effects of the transgenes must be carefully and critically assessed independently and monitored by several scientific groups rigorously for a long time. Such a mammoth effort certainly reduces the risk of transgenes and their potential environmental hazards if any.
- GM crops or their products that are substantially equivalent with their counterparts can only be given approval for commercialization, and such GM crops must be evaluated both spatially and temporally in a cost-effective manner.
- More importantly, the methods of gene transfer or modifications should reduce the risks and improve benefits to ecosystems. The methods of tissue culture can cause genetic variation (somaclonal variation), hence must be avoided for gene transfer. Instead, the technology of gene editing like CRISPR-Cas9 may be a superior way of gene editing for getting required benefits.
- The change in nutritional characteristics in GM crops as compared to their counter parts should be evaluated carefully over a period of time (Pauwels et al. 2015).
- A detailed study on the toxicity and allerginicity of GM foods should be performed (Domingo 2007; De Santis et al. 2018).
- Also, transgenic events with single-gene insertions are preferred to avoid gene silencing in subsequent generations and for subsequent safety assessment (Tiwari and Singh 2018). In several labs, such a procedure is being followed which can ensure us stable integration and expression of the transgenes.
- An intensive research must also be carried out in different countries if gene stacking or trait stacking is leading to the sale of GM seeds that are exorbitant than what resource-poor farmers can afford.
- The labelling of GM foods should be mandatory (Huffman and McCluskey 2017; Kamle et al. 2017; Moghissi et al. 2018).
- GM traceability which enables tracking of GM food or feed products at all stages of the supply chain should be considered (Giraldo et al. 2019).
- If better technologies such as CRISPR-Cas9 are adapted, then it must be debated publicly, and consensus opinion must be arrived. The consequences of such genetic tinkering on ethical, legal and social issues must be resolved and addressed properly before the release of GM foods.
- Besides product safety, policymakers should also carefully address all issues related to technology governance, be it a private or public sector, and also competing interests of stakeholders and associated trade-offs.

6 Conclusions

The ability to isolate and insert genes of interest into crop plants at will with tissue-specific promoters is a milestone. We now have the potential tools to introduce multiple genes into the host plant of interest that can affect polygenic traits. Newer protocols with improved efficiency and single gene insertions have been developed for a majority of crop plants and irrespective of the genotype. Despite the generation of diverse GM crops with remarkable improvement in tolerance to biotic and abiotic stresses, herbicide tolerance and nutritional quality improvement, we do not grow many GM crops in the field conditions. Further, governments do not have the resolve to strictly follow the regulatory systems so as to take care of the ecosystems. General public has been opposing the introduction of GM crops tooth and nail, but without much debate on safety issues. WHO has been assessing the human health risks due to consumption of GM food, but could not find any potential toxicological risks. The results obtained through several independent projects, and nearly four decades of transgenic research data generated in both public and private sectors around the world revealed that GM foods *per se* are not risky in comparison with plant breeding technologies (European Commission 2010). These facts infer that genetic engineering technologies, and the GM crops are not risky to animal and human health and do not cause any harm to the environment. It is time for us to review the current and future commercial status of GM crops and their benefits/risks to the society at large. The situation in Europe is totally different since we have dichotomy of experience. Paradoxically, they do not grow GM crops but import the same from other countries. Our attitude towards GM crops must change in future since opportunities and benefits abound with GM crops, but with a note of caution about environmental safety, ecological security and animal and human health risks.

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Genetic Improvement of Rice for Food and Nutritional Security

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Abstract

Rice is a staple food consumed by almost half of the world's population. However, in a natural environment, like any other plant, rice is exposed to various abiotic stresses such as salinity, drought, and high temperature, which in turn affect its yield. Therefore, to meet the demand of the world's growing population, it is imperative for scientists to come up with novel strategies of combating these abiotic stresses. Over the years, transgenic rice showing improved performance under stresses such as salinity, drought, and cold have been developed using genetic engineering approaches. Additionally, scientists have also developed rice that has higher nutrient content such as, golden rice, folate-biofortified rice, iron-fortified rice, and zinc-fortified rice. In this chapter, we discuss how plants respond to heat, cold, salinity, drought, and flooding stress with an emphasis on the physiological, biochemical, and molecular mechanisms of stress tolerance. Further, we also present a few representative success stories where attempts have been made towards improving the nutritional value or for enhancing stress tolerance in rice. This information may help in promoting the interdisciplinary studies designed to assess the stress-responsive genes and their role under various abiotic stresses along with a target of improving the nutritional value in rice.

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Abiotic stress · Tolerance · Genetically modified rice · Golden rice · Nutrition

1 Introduction

Practices of crop selection and breeding to enhance yields have been adopted since the beginning of the agricultural era which dates back to about 10,000 years (Voss-Fels et al. 2019). The mode of choosing for higher and better crops is followed mostly to compensate the increase in demands with the rise in population. It is estimated that by the year 2050, the world population would reach nine billion (https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html, accessed on 5 March 2020). To feed this growing population, it is estimated that the global food production should increase by 44 million tons each year (Qaim 2009). However, with the changing climate causing severe environmental degradation leading to drastic reduction in soil fertility and the severity of drought, salinity, high temperature, cold, etc. becoming more prevailing (Pareek et al. 2020), a challenge is laid before us to produce such a high volume of crops.

In spite of high carbohydrates and sugar content, the nutritional value for most of the rice types are found to be low (Gregory et al. 2017). At the same time, nearly half of the world's population is dependent on rice as a staple food. In Asia alone, it is estimated that approximately 1.3 billion people consume rice every day (Maclean et al. 2013). Therefore, if rice with high nutritional contents can be developed, more than half of the world's population will be free from malnutrition. This is one of the reasons why rice draws attention of crop scientists who are working towards food and nutritional security missions of the countries.

Genetic engineering for crop development was introduced in the early 1980s (Wieczorek and Wright 2012), and by mid-1990s, the first genetically modified (GM) plant, tobacco with resistant to herbicide was released in France and USA. However, the first commercially available GM food, tomato with delayed ripening was released in 1994 from the University of California, Agriculture and Natural Resources by the name *Flavr Savr* (Bruening and Lyons 2000). Subsequently, other crops such as maize, canola, and soybean with various traits were also genetically modified and released commercially in several countries including Argentina, Canada, and the USA (Anderson et al. 2004). Crops developed through genetic engineering do not vary much from those pursued by conventional breeding. However, the benefit of using genetic engineering over traditional breeding is that it is a targeted approach and takes shorter time to develop the desired traits. Through this technique, several traits that were impossible through conventional breeding have also been developed (Qaim 2009; Zafar et al. 2019).

Keeping in mind the importance of rice as a staple food crop, there is an urgent need to have food security along with nutritional security with a clear focus on this crop. In this chapter, we present a few representative success stories targeting the development of genetically modified rice for nutrient enrichment and enhanced tolerance to various abiotic stresses. Additionally, we also briefly touch on the economic benefits of GM rice.

2 Traditional Methods to Develop the New Rice Types

The conventional approach to develop improved rice varieties primarily relies on the tools of plant breeding. Several novel genotypes carrying a desired character have been generated by crossing different parental lines (Schaart et al. 2016; Hickey et al. 2017). One of the best example in this category is the development of dwarf wheat genotypes during green revolution (1960s). This dwarf variety of wheat was high yielding and resistant to lodging (Swaminathan 2000). After this successful breakthrough, several plant breeders and scientists continued further work on developing rice varieties through conventional breeding approaches that resulted in improved grain quality (nutritional) and disease resistance (Breseghello and Coelho 2013).

Broadly, conventional breeding can be categorized as follows: (1) *Pedigree breeding*, in this approach, two contrasting parental lines are crossed to generate segregating populations, and cultivar with desirable characteristics is selected. This method can be applied only to self-pollinating species for developing suitable quantitative traits like disease resistance and plant architecture-related traits like shape or color of plant parts (Crossa et al. 2017). (2) *Ideotype breeding*, this approach is based on the hypothesis that complex traits can be improved by modifying the individual traits that govern specified phenotype. Ideotype breeding addresses the strategy to improve the pedigree method, so that yield can be promoted (Rasmusson 1987; Peng et al. 2008). (3) *Population breeding*, this approach focuses on the methods designed for the intermating population so that their phenotypic performance can be improved. To achieve this goal, frequency of favorable alleles is increased that is controlling the desirable traits (Breseghello and Coelho 2013). (4) *Hybrid breeding,* in this breeding technique, two homozygotic but genetically different parental lines are crossed resulting in the development of heterozygotic offspring (Cui et al. 2020). Using these breeding techniques, considerable efforts have been made to develop rice varieties that have high nutrient content and are tolerant to stress. One of the recent examples is the development of “the new plant type” (NPT), a rice variety, by a group of scientists from the International Rice Research Institute (IRRI), Philippines, which produces more than 200 grains per panicle. This variety has dark green leaves that are erect and thick, whereas the panicles are larger and stronger than the parental lines and thus can withstand the weight of the grains (Uphoff et al. 2015).

3 Raising Genetically Modified Rice with Nutrient Enrichment

Over the years, several successful attempts have been made to improve the nutritional content of crops through genetic engineering (Ye et al. 2000; Akhtar et al. 2013). For brevity sake, some of these success stories that have left an impact at a global scale against the fight for malnutrition and hunger are summarily presented in Table 1.

Table 1 Representative success stories for the genetic modification in rice for the selected traits

GM rice	Target gene(s)	Method of transformation	Trait improvement	Reference(s)
Genetically modified rice for nutritional enrichment				
Golden rice	*Psy* and *CrtI*	*Agrobacterium*-mediated transformation	Provitamin A enrichment	Ye et al. (2000)
Folate-fortified rice	*GTPCHI* and *ADCS*	*Agrobacterium*-mediated transformation	Folate biosynthesis and enrichment	Storozhenko et al. (2007)
Iron-fortified rice	*AtNAS1* and *Pvferritin*	Biolistic-mediated transformation	Iron enrichment	Vasconcelos et al. (2003)
Zinc-fortified rice	*OsNAS1*, *OsNAS2*, and *OsNAS3*	Biolistic-mediated transformation; *Agrobacterium*-mediated transformation	Zinc enrichment	Vasconcelos et al. (2003), Johnson et al. (2011)
Genetically modified rice for enhanced stress tolerance				
Glyphosate-tolerant rice	*CP4-EPSPS*	*Agrobacterium*-mediated transformation	Glyphosate tolerance	Chhapekar et al. (2015)
BT rice	*CryI*	Electroporation transformation	Resistance to Lepidopteran pests	Fujimoto et al. (1993)
Salinity-tolerant rice	*SOS1, SERF1, SOS2, SOS3, STRK, CNAtr, MYB2, MnSOD, GS, katE, ADC, codA, SAMDC, NHX1,* Os*KAT1*, Os*Cyp2* Os*HBP1b*, Os*GATA8*, Os*PGK2-P*, Bj*GLY I*, and Os*GLY II*	*Agrobacterium*-mediated transformation	Salinity tolerance	Tanaka et al. (1999), Hoshida et al. (2000), Roy and Wu (2001), Mohanty et al. (2002), Ma et al. (2005), Nagamiya et al. (2007), Verma et al. (2007), Singh et al. (2008), Kumari et al. (2009), Joshi et al. (2016), Lakra et al. (2015), Gupta et al. (2018), Nutan et al. (2020)
Drought-tolerant rice	*P5CS2, ICE1, HOS1, OsNAC14, COX1, PKDP, bZIP1, AP2-EREBP, Hsp20, DREB* family, *ABF3, SNAC1, COC1*, and *OsLG3*	*Agrobacterium*-mediated transformation	Drought tolerance	Singh et al. (2008), Saakre et al. (2017), Shim et al. (2018), Xiong et al. (2018)

(continued)