

Status of Transgenic Cereal Crops: A Review

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Abstract

The era of transgenic plants, begin with the field trials of genetically engineered tobacco plants in early eighties. The success story for cereal crops took almost a decade after first transgenic plant development. The first transgenic cereal crop released commercially was *Bt* maize. The success of insect pest tolerant maize and rice varieties witnessed the approval of this promising technology. Scientific institutions and private companies concerted their efforts in terms of time and capital investment for this demanding field. This led to many landmark achievements in all the challenging areas where conventional breeding could not make much progress in terms of economic benefits. Genetically engineered plants offer higher yields, better adaptability, less reliance on chemicals, and greater nutritional value. The wider acceptability of genetically modified crops particularly cereals will strengthen the food security issues for combating the fear of hunger outraging with increasing population. This review summarizes the landmark achievements, advancements, commercialization and the global status of genetically modified cereals particularly maize, rice and wheat.

Keywords: Transgenics; *Bt* maize; Insect pest; Herbicide; Abiotic stress; Nutritional enhancement; Phytoremediation; Global status; *Bacillus thuringiensis*

Introduction

The world population is on rise and will reach 9.5 billion mark by year 2050 as per current statistics. Global food production will need to increase by 70%, which means global rice and maize production must be two fold from same available resources [1]. The natural resources are shrinking and demand for basic life essentials swelling every day. Alternative sources to increase food, water and energy along with innovative approaches to curb their losses is utmost important for sustainable life on planet earth. Technological advances in past strengthened our agricultural systems to vanish all predictions of food insufficiency owing to ever growing population pressure. Cereals grains are basic source of food and energy. The major cereals; wheat, maize, rice, barley, sorghum, oats, rye, and millets provide 56% of the food energy and 50% of the protein consumed on earth [2]. The demand for cereals will be two fold by 2050 to meet the requirements of massive population (Figure 1). In early 1980s, genetic transformation of crop plants based on recombinant DNA technology was started and this

offered advantage of transferring novel gene(s) across taxonomic boundaries unlike conventional breeding.

Transgenic plants are integral part of present day agriculture and in fact, in some regions it is difficult to differentiate transgenics from conventionally grown varieties. The areas under Genetically Modified (GM) crops increased since their inception and are currently grown over 28 countries. Table 1 shows the commercialized transgenic cereal crops for improved traits and their cultivation worldwide. There is an unprecedented 100-fold increase in biotech crops area from 1.7 million hectares in 1996 (first commercialized biotech cereal crop) to 170 million hectares in 2012 [3,4]. This made biotech crops the fastest adopted crop technology in recent history. The success story for genetically modified cereals crops is little tangible than other crops of economic importance. It took long time for transgenic development in cereals due to needs of techniques for stable transgene production, horizontal gene transfer and issues regarding acceptability of GM cereal grains. In this review, the central focus is on transgenic technology used and developed in major cereal crops including rice, maize and wheat since their inception is discussed.

Insect Pest Resistance in GM Cereal Crops

Crop plants have been genetically engineered to produce proteins from the soil bacteria *Bacillus thuringiensis* (*Bt*) that make plants resistant to certain lepidopteran and coleopteran species. *Bt* toxins are highly specific against insects without affecting predators and other beneficial insects [5,6]. *Bt* crops produce *Bt* toxins in most of their tissues. These *Bt* toxins kill susceptible insects feeding on these *Bt* crops. This means that *Bt* crops are especially useful for controlling pests that feed inside plants and that cannot be killed readily by sprays such as the European corn borer (*Ostrinia nubilalis*), which bores into stems.

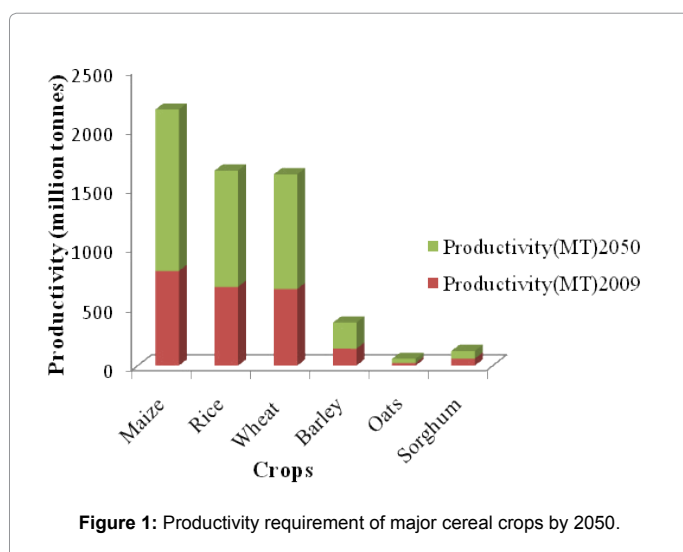


Figure 1: Productivity requirement of major cereal crops by 2050.

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Crop	Trait ¹	Organization/Company	Countries
32138 SPT maintainer Maize	PCS	Dupont	USA
Bt Shanyou 63 Rice	IR	Huazhong Agricultural University (China)	China
Bt Xtra™ Maize	IR	Monsanto	Australia, Canada, Japan, New Zealand, Philippines, South Korea, Taiwan, USA
Enlist™ Maize	HT	Dow AgroSciences LLC	Australia, Canada, Mexico, New Zealand, South Africa, Taiwan, USA
Enogen™ Maize	MPQ	Syngenta	Australia, Canada, Japan, Mexico, New Zealand, Philippines, Russian Federation, South Korea, Taiwan, USA
Genuity® DroughtGard™ Maize	AST	Monsanto	Australia, Canada, Japan, Mexico, New Zealand, Taiwan, USA
Herculex XTRA™ Maize	HT, IR	Dow AgroSciences LLC and DuPont	Canada, EU, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, Turkey, USA
Huahui-1 Rice	IR	Huazhong Agricultural University (China)	China
InVigor™ Maize	PCS	Bayer Crop Science	Canada, USA
Mavera™ YieldGard™ Maize	MPQ	Renessen LLC and Monsanto	Japan, Mexico, USA
NaturGard KnockOut™, Maximizer™ Maize	HT, IR	Syngenta	Argentina
Optimum™ GAT™ Maize	HT	DuPont	Argentina
Power Core™ Maize	HT, IR	Monsanto and Dow AgroSciences LLC	Argentina
Roundup Ready™ 2 Maize	HT	Monsanto	Argentina
Roundup Ready™ Wheat	HT	University of Florida	Australia, Colombia, New Zealand, USA
Starlink™ Maize	IR	Bayer Crop Science	USA
YieldGard™ Plus Maize	IR	Monsanto	EU, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, USA

¹Abbreviations: AST- Abiotic Stress Tolerance; DR- Disease Resistance; HT- Herbicide Tolerance; IR- Insect Resistance; MPQ- Modified Product Quality; PCS- Pollination Control System

²Adapted from [3]

Table 1: Description of commercially released transgenic cereal crops worldwide².

Bt toxins are also known as *Cry* toxins because they exist as crystals inside the bacterium. Full-length *Cry* toxins are inactive until cleaved to generate their active forms in the insect midgut [7,8]. Binding of activated forms of *Cry* toxins to receptors in the midgut is generally believed to be essential for toxicity. The developed model considers that activated toxins after binding to midgut receptors form oligomers create pores in midgut membranes causing gut leakage ultimately killing the larvae [9,10]. Before *Bt* crops were developed, *Bt* sprays were used for insect control and are still used extensively [11]. The long-term history of the use of *Bt* sprays allowed the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) to consider decades of human exposure in assessing human safety before approving *Bt* crops for commercial use. Further, toxicity and allergenicity tests were conducted on different kinds of naturally occurring *Bt* toxins. These tests and the history of spraying *Bt* toxins on food crops led to the conclusion that *Bt* corn is as safe as its conventional bred varieties and therefore, would not adversely affect human and animal health or the environment [12].

Bt crop cultivation reduces the use of chemical insecticides and thus provides environmental and economic benefits leading to sustainable agricultural production. The United States of America took lead in 1996 to commercially release *Bt* maize followed by Canada in 1997 thereafter, adoption of transgenic maize in Argentina, South Africa, Spain and France. The area under *Bt* maize was extended to 35 million hectares in more than 15 countries in the year 2009. The biggest *Bt* maize areas are found in the USA, Argentina, South Africa, Canada and the Philippines [13]. *Bt* maize offers both economic and environmental benefits, and grower responses indicated an awareness of both types of these benefits. There was cumulative decrease of insecticide active ingredient (a.i.) to the amount of 35% (29.9 million kg) on *Bt* maize globally [14]. Much of the reduction in insecticide a.i. was probably due to coleopteran active *Bt* maize, as insecticides used against *Diabrotica spp.* comprise 25-30% of the global total in maize [15]. *Bt* crop cultivation also opened the goal for sustainable agriculture by supporting the non target invertebrate population (insects, mites, spiders and related species) in *Bt* corn and

cotton fields than conventional crop fields managed with insecticides [16]. Benefits of *Bt* crops have also been well documented in less-developed countries. For example, Chinese and Indian farmers growing genetically engineered crops were able to dramatically reduce their use of insecticides [17-19].

The extensive use of any insecticides or genetically engineered crops increase chances of developing selection pressure led to evolution of new insect resistances. For example, the diamond black moth (*Plutella xylostella*) has evolved resistance to *Bt* toxins under open field conditions [20]. Experience with *Bt* crops showed that resistance in these crops remained effective against most pests for more than a decade [21,22]. Several strategies were suggested to maintain durability of resistance in *Bt* crops [23]. The refuge strategy involves growing of crop plants without *Bt* toxins to promote survival of susceptible insects that helped to delay evolution of pest resistance to *Bt* crops [22]. The refuges allow populations of non-resistant insect to multiply and breed with any insects that may have developed resistances [24,25]. The other approaches developed include pyramiding of *Cry* genes, modifying the *Cry* genes and using different promoters to alter the level and site of expression to reduce the risk of new insect resistances. The various practices were developed to prevent insect resistances includes different *Cry* genes in different varieties, pyramiding of *Cry* genes and refuge crop planting for *Bt* maize (Table 2).

Rice is most important food crop and feeds more than half of the world's population. Rice crop yield losses mainly occurred due to infestation of stem borers and estimated to be 5-10% [26]. In other study, losses caused by rice stem borers in spite of insecticides application were estimated to be 3.1% of national rice yield [27]. Application of chemical pesticides is the major method to control insects in rice crop. The excessive use of these insecticides not only increased production cost but also pollutes environment and threatens human health. Conventional plant breeding approaches for developing resistant varieties were not successful due to non-availability of effective sources of resistance against striped stem borer (*Chilo suppressalis*),

Transgenic Maize	Bt gene	Trade name	Pests controlled or suppressed	Refuge requirement
Bt 11	<i>CryIAb</i>	YieldGard Agrisure	ECB, SWCB, FAW, CEW	20% non-Bt refuge within ¼ to ½ mile of Bt fields
MON 810	<i>CryIAb</i>	YieldGard	ECB, SWCB, FAW, CEW	20% non-Bt refuge within ¼ to ½ mile of Bt fields
TC 1507	<i>CryIF</i>	Herculex 1	ECB, SWCB, BCW, FAW, CEW	20% non-Bt refuge within ¼ to ½ mile of Bt fields
MON 863	<i>Cry3Bb1</i>	YieldGard Rootworm	CRW	20% non-Bt RW refuge in or immediately adjacent to Bt RW field
MON 863 + Mon 810	<i>CryIAb + Cry3Bb1</i>	YieldGard Plus	ECB, SWCB, CRW	20% non-Bt refuge in or immediately adjacent to Bt field
DAS 59122-7	<i>Cry34Ab1 + Cry35Ab1</i>	Herculex RW	CRW	20% non-Bt RW refuge in or immediately adjacent to Bt RW field
TC 1507 + DAS 59122-7	<i>CryIF + Cry34Ab1 + Cry35Ab1</i>	Herculex XTRA	ECB, SWCB, BCW, FAW, CE, CRW	20% non-Bt refuge in or immediately adjacent to Bt field
MIR 604	<i>mCry3A</i>	Agrisure RW	CRW	20% non-Bt RW refuge immediately adjacent to Bt RW field
Bt 11 + MIR604	<i>Cry1Ab + mCry3A</i>	Agrisure CB/RW	ECB, SWCB, FAW, CEW, CRW	20% non-Bt refuge in or immediately adjacent to Bt field
Mon 88017	<i>Cry3Bb1</i>	YieldGard VT RW	CRW	20% non-Bt RW refuge in or immediately adjacent to Bt RW field
Mon 810 + Mon 88017	<i>Cry1Ab + Cry3Bb1</i>	YieldGard VT Triple	ECB, SWCB, CRW	20% non-Bt refuge in or immediately adjacent to Bt field
TC 1507 + DAS 59122-7 + Mon 89034 + Mon 88017	<i>CryIF + Cry34Ab1 + Cry35Ab1 + Cry3Bb1 + Cry1A.105 + Cry2Ab</i>	SmartStax	ECB, SWCB, FAW, CEW, CRW	5% non-Bt refuge in or immediately adjacent to Bt field
TC 1507 + DAS 59122-7 and TC1507	90% <i>CryIF + Cry34Ab1 + Cry35Ab1</i> and 10% <i>CryIF</i> seed	AcreMax 1	ECB, SWCB, CRW	No external refuge for RW, but a 20% corn borer refuge needed within ¼ to ½ mile
DAS 59122-7 and non-Bt seed	90% <i>Cry34Ab1 + Cry35Ab1</i> and 10% non-Bt seed	AcreMax RW	CRW	No external refuge needed
Mir162 and Bt11	<i>VIP and CryIAb</i>	Agrisure Viptera 3110	ECB, SWCB, BCW, FAW, CEW	20% non-Bt refuge needed within 1/4 to 1/2 mile
Mir162, MIR604 and Bt11	<i>VIP, mCry3A and CryIAb</i>	Agrisure Viptera 3111	ECB, SWCB, BCW, FAW, CEW, CRW	20% non-Bt refuge in or immediately adjacent to Bt field
TC1507 and Mon810	<i>Cry1F and CryIAb</i>	Optimum Intrasect	ECB, SWCB, BCW, FAW, CEW	5% non-Bt block or strip refuge

Abbreviations: ECB- European Corn Borers; SWCB- Southwestern Corn Borers; FAW- Fall Army Worm; CEW- Corn Earworm; BCW- Black Cutworm; CRW -Corn Rootworms

Table 2: Commercial Bt corn practices for longer and durable insect resistance.

Yellow stem borer (*Tryporyza incertulas*) and leaf folder (*Cnathalocrocis medinalis*). Many useful insect resistant genes have been identified and isolated from plants, animals and from microorganisms. Bt genes have been successfully transferred and expressed in different rice varieties (Table 3). Transgenic insect resistant rice developed using these genes have been tested under field conditions, which showed resistance and yield advantage. The most frequently used Bt genes are *Cry1A*, *Cry1Ab* and *Cry1Ac* and *Cry1Ab/Ac* fusion gene (Table 3). These Bt rice lines showed resistance against striped stem borer, yellow stem borer and leaf folder. Field evaluation of transgenic rice harbouring a *Cry1Ab/1Ac*, Minghui 63 and its hybrid Bt Shanyou 63 showed insect resistance without any insecticidal spray indicated huge value of Bt rice. However, many insects developed resistance to Bt toxins in green house or laboratory conditions and were able to survive on Bt crops directing the threat that insects have potential to evolve resistance against Bt crops [23]. Resistance management is major technical challenge for Bt rice. Developing transgenic varieties containing two appropriate toxin genes with high dose of expression need to be research priority [28,29]. However, the two Bt toxin in combination must bind to different receptor sites on insect gut cells to avoid occurrence of cross resistance. Studies Showed that *Cry1A*, *Cry1C* and *Cry2A* are suitable to combine and its very unlikely that insect develop a cross resistance to these combinations due to low protein sequence homology with each other [30,31]. Bt rice offers the potential to generate benefits of US\$4 billion annually with an average yield increase of 8 percent and 80% decrease in insecticide use [32]. The first insect resistant genetically modified rice transformation was done in 1989 in China however; it took 30 years for commercial release of transgenic rice after getting biosafety clearance from Chinese Ministry of Agriculture for commercial production of two *Cry1Ab/Ac Bacillus thuringiensis* (Bt) lines against lepidopteron

complex [33]. Experience has shown the benefits of transgenic insect-resistant crops in terms of increased yields, reduced chemical inputs, improved farmer and consumer health. However, the possibility of pest resistance development and damage to non-target species necessitates proper deployment of transgenic plants expressing insecticidal genes.

Disease Resistance in GM Cereal Crops

A large number of fungal, bacterial, and viral pathogens attack cereal crops. Enhancing the disease resistance of cereal crops to these pathogens through the development of transgenic plants is a viable approach to avoid losses in these crops. The cloning of disease resistance genes and the knowledge of the signal transduction components of the hypersensitive response and systemic acquired resistance pathways has greatly increased the diversity of options available for transgenic disease resistance. In wheat, Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, is one of the most important diseases of wheat worldwide, resulting in yield losses and mycotoxin contamination. The fungal pathogens are known to secrete Polygalacturonases (PGs) during the early stages of infection, and plants have evolved Polygalacturonase-Inhibiting Proteins (PGIPs) to restrict pectin degradation during fungal infection. Transgenic wheat plants expressing the bean *PvPGIP2* in their flowers showed significant reduction of symptoms on inoculation with *F. graminearum* [52]. In other study, transgenic wheat and Barley plants showing resistance against head blight was obtained by expressing bovine lactoferrin cDNA. The bovine lactoferrin gene is a broad-spectrum antimicrobial gene [53]. In Rice, the blast (*Magnaporthe grisea*), bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*) and sheath blight (*Rhizoctonia solani*) are the most serious constraints for high productivity. Several strategies have been established for developing and improving rice resistance against fungal and bacterial diseases through

Gene	Promoter	Cultivar	Reference
<i>cry1Ab</i>	Ubiquitin	KMD1, KMD2	[34,35]
<i>cry1Ac</i>	Ubiquitin	Elite Eyi 105,Bengal,	[36]
<i>cry1Ac</i>	Ubiquitin	IR64, Pusa Basmati-1, Karnal Local	[37]
<i>cry1Ab, cry1Ac</i>	Ubiquitin	Basmati370	[38]
<i>cry1Ab</i>	CaMV35S	IR58	[39]
<i>Cry1Ab</i>	CaMV35S, Actin-1,pith tissue specific, PEPC	IR72, IR64, CBII, Taipei-309, IR 68899B, MH-63-63, IR5 1500-AC11, Vaideh-1, IRR1-npt	[40]
<i>cry1Ac</i>	PEPC	Tarom Molaii	[41]
<i>cry1Ac</i>	Ubiquitin	IR64	[42]
<i>cry1Ab</i>	CaMV35S	Vaidehi, TCA-48	[43]
<i>Cry2A</i>	CaMV35S	Basmati-370, M-7	[27]
<i>Cry1Ab, cry1Ac</i>	Ubiquitin	Kaybonnet, Nipponbare, Zhong8215, 93VA, ZAU16, 91RM, T8340, Pin92-528, T90502	[44]
<i>Cry1Ab</i>	CaMV35S	Taipie-309	[45]
<i>Cry1Ab</i>	Pollen specific, Ubiquitin, PEPC	Basmati370	[46]
<i>Cry1B</i>	Ubiquitin	Ariete, Senia	[47]
<i>Cry1B</i>	Maize proteinase inhibitor	Ariete	[48]
<i>Cry1Ab/ cry1Ac hybrid gene</i>	Actin-1	CMS restores Minghui63, Shanyou 63	[49]

¹Adapted from [50,51]

Table 3: *Bt* rice lines with different Crygenes for resistance against lepidopteron insects¹.

transgenic approaches. Genes encoding chitinase or 1, 3-glucanase from plants and microbes have been extensively used in generation of transgenic rice resistant to fungal pathogens [54]. Transgenic plants constitutively expressing the *Gns1* gene, encoding a 1, 3:1, 4- glucanase accumulated *Gns1* protein up to 0.1% of total soluble protein in leaves. The *Gns1* overexpressing transgenic plants developed resistant-type lesions on the inoculation with virulent *Magnaporthe grisea* [55]. Transgenic plants which constitutively expressed a rice class-I chitinase gene, *Cht-2* or *Cht-3*, showed significant resistance against two races of *Magnaporthe grisea* [56]. Hydrolytic enzymes of microbial origin have also been demonstrated to be effective in engineering rice with resistance against fungal pathogens. A bacterial family 19 chitinase *ChiC* from *Streptomyces griseus* showed clear inhibition on fungal hyphal extension. Ninety percent of transgenic rice plants expressing *ChiC* had higher resistance against *M. grisea* than non-transgenic plants. Disease resistance in the transgenic plants was correlated with the *ChiC* expression levels [57]. Antifungal Proteins (AFP) from *Aspergillus giganteus* have been reported to possess *in vitro* antifungal activity against various economically important fungal pathogens including *M. grisea*. Transgenic rice with constitutive expression of AFP protein showed stable inheritance of the transgene without any effect on plant morphology, growth and development [58]. The AFP protein prepared from leaves of transgenic plants exhibited inhibitory effect on *in vitro* growth of *M. grisea* and the transgenic plants showed enhanced resistance to the blast disease [59].

Transgenic rice plants that constitutively express the wheat puroindoline genes *PinA* and/or *PinB* were produced. Puroindolines in leaf extracts of the transgenic plants reduced *in vitro* growth of *M. grisea* and *R. solani* by 35 - 50%. Transgenic rice expressing *PinA* and/or *PinB* showed significantly increased resistance to *M. grisea* and *R. solani* [60].

Transgenic rice plants expressing *AtNPR1* showed enhanced disease resistance against *M. grisea* and *Xanthomonas oryzae pv. oryzae* by priming the expression of Salicylic acid-responsive endogenous genes, such as the *PR1b*, *PR5*, *PR10* and *PBZ1* [61-63]. The rice genome contains five *NRI*-like genes and three of them, *OsNPR1*, *OsNPR2* and *OsNPR3*, were induced by infection of *Xanthomonas oryzae pv. oryzae* and *M. grisea*. *OsNPR1* is the rice orthologue of Arabidopsis *NPR1* gene. Overexpression of *OsNPR1* conferred disease resistance to bacterial

blight, but also enhanced herbivore susceptibility in transgenic rice plants [64,65]. *OsNPR1* might mediate antagonistic cross talk between the Salicylic Acid (SA) and Jasmonic Acid (JA) dependent pathways and thus provides a practical approach for engineering broad-spectrum disease resistance in rice [65]. Constitutive expression of pathogen-inducible genes encoding transcriptional factors enhances disease resistance against *M. grisea* through activation of expression of many defense related genes [66]. On other hand, genetic manipulation of the JA biosynthesis pathway has also been shown to improve rice disease resistance against fungal and bacterial pathogens. It was found that transgenic rice plants over expressing a pathogen-inducible *OsAOS2* gene, which encodes an allene oxide synthase, a key enzyme in the JA biosynthetic pathway, accumulated higher levels of JA, up-regulated expression of PR genes and increased resistance to *M. grisea* infection [67]. In spite of large amount of efforts dedicated and success achieved in developing and testing transgenics for disease resistance in cereal crops, there is no commercial release so far.

Herbicide Tolerance in GM Cereal Crops

Weeds compete with plants by robbing water, light, space, and soil nutrients. They can produce allelopathic substances that are toxic to crop plants. These unwanted plants often serve as hosts for crop diseases; and provide shelter for insects and diseases to overwinter. Weeds cause enormous economic losses in crops and the loss was estimated approximately \$6 billion per annum in United States (US) [68]. A number of herbicides were used to kill these noxious weeds. The herbicide available for killing these weeds could be selective or nonselective in their mode of action.

Two most important non-selective herbicides used are glyphosate and glufosinate. The mechanism of glyphosate lies in the inhibition of Enolpyruvyl Shikimate-3-Phosphate (EPSP) synthase enzyme [69]. This enzyme is involved in shikimate pathway which is responsible for making aromatic amino acids tryptophan, tyrosine, and phenylalanine and thus in turn interferes with the growth of weedy plants. The advantage of using herbicides targeting shikimate pathway strengthened by the fact that this pathway does not exist in animals therefore; this herbicide is safe for insects, animals, humans, birds and fish. The other herbicide group, glufosinate inhibits glutamine synthetase [70]. This inhibition blocks the conversion of glutamate and ammonia

to glutamine. This led to accumulation of ammonia which destroys cells [71] and inhibits photosystem I and II reactions thus results in uncoupling of photophosphorylation [72].

Genetically modified Herbicide Tolerant (HT) plants were made against these non-selective herbicides. A number of gene transfer methods were tried to develop transgenic plants tolerant to herbicides. The first successful herbicide tolerant transgenic tobacco was made using *Agrobacterium* gene transfer method [73]. *Agrobacterium* transfer methodology was not successful in cereals in the beginning. Gene transfer becomes a reality with the development of direct gene transfer methods and the most successful method was particle bombardment [74-80]. Genes for resistance to chlorsulphuron or phosphinothricin in maize using high velocity micro projectiles were discussed in many studies [74,75,81].

Monsanto lead the development of first transgenic glyphosate resistant corn (roundup ready) and this was tested at different locations of US [82], which became commercial success in the year 1998. This followed the release of many commercial cultivars with tolerance to other herbicides for instance, Liberty Link Corn against glufosinate. Recently transgenic maize expressing Dicamba Monooxygenase (DMO) linked with Chloroplast Peptide (CTP) showed tolerance to dicamba at pre and post emergence crop stages [83]. Later stacking of traits in single cultivar was used to control weeds and insects in the cereal crops for instance; transgenic maize expressing two *Cry* genes *Cry34Ab1*, *Cry35Ab1* pyramided with *PAT* (phosphinothricin acetyl transferase) genes for herbicide tolerance was recently developed by Pioneer [84]. Research efforts for identifying genes, potentially regenerable explants and novel gene transfer methods in past two decades witnessed more than 50% herbicide tolerant varieties over the total corn varieties grown in US [85].

Rice is other staple food crop that attracted the attention of research groups to target for herbicide tolerance. Extensive research efforts have been made for developing herbicide tolerant rice with bar gene against glufosinate (Basta) using particle acceleration method [76,86,87]. This was trailed by utilizing number of tolerant genes against non-selective herbicides using various transgenic approaches [88]. Table 4 describes the major developing transgenic cereals developed against various group of herbicides. The major strategies for developing commercial herbicide tolerant rice target three pathways. These include shikimate pathway which lead to development of Roundup Ready® rice, glutamine biosynthesis pathway leading to Liberty Link® and branched chain amino acid synthesis leading to the development of Clearfield®. Clearfield rice is non-transgenic whereas Roundup ready and Liberty Link rice are transgenic [89]. Recent work for developing transgenic rice plants with enhanced melatonin levels provides protection against oxidative stress developed in response to herbicide application [90].

After the success of herbicide tolerant maize and rice, the

Crop	Herbicide against which transgenic plants were made	Reference
Rice	Bialaphos	[76]
	Methotrexate	[86]
	Glufosinate ammonium	[87,93]
	Oxyfluorfen	[94,95]
Maize	Chlorsulphuron Phosphinothricin	[74,75]
	Glyphosate	[96]
	Glufosinate	[97]
Wheat	Glufosinate ammonium	[98,99]

Table 4: Transgenics developed against various herbicide groups in cereal crops .

development of roundup ready wheat took place in the year 1997. However, the approval from Food and Drug Administration (FDA) regarding safety of roundup ready wheat delayed its release. Monsanto got approval in 2004 to release glyphosate-resistant roundup ready wheat with *CP4/EPSPS* gene, but due to concerns regarding international trade of genetically modified wheat, this approval was suspended in 2005 [91].

The most important benefit of transgenic herbicide tolerant plants is reduced crop injury. Non-selective herbicides such as glyphosate and glufosinate aid in widening the spectrum of weed control. Systemic activity of glyphosate favors perennial weeds control. The structural property of these herbicides helps in tightening of the organic particles of the soil hence, showing no residual activity in soil [92] and thus facilitates flexible crop rotation. These herbicides have lower toxicity to humans and animals. They are easily decomposed with in the soil thus leaching; contamination to ground water and toxicity to wildlife can be

Gene	Pathway/Function	Crop	Reference
<i>AhBADH</i>	Osmoregulation	Wheat	[124]
<i>bet A</i>	Osmoregulation	Wheat	[125]
<i>OsbZIP72</i>	ABA Response	Rice	[126]
<i>SQS</i>	ABA synthesis	Rice	[127]
<i>OsDST</i>	ABA sensing	Rice	[128]
<i>TsCBF1</i>	AP2/ERF family transcription factor	Maize	[129]
<i>JERF1</i>	AP2/ERF family transcription factor	Rice	[130]
<i>JERF3</i>	AP2/ERF family transcription factor	Rice	[131]
<i>TSRF1</i>	AP2/ERF family transcription factor	Rice	[132]
<i>OsDREB2A</i>	AP2/ERF family transcription factor	Rice	[133]
<i>ZmCBF3</i>	AP2/ERF family transcription factor	Rice	[134]
<i>GhDREB</i>	AP2/ERF family transcription factor	Rice	[135]
<i>TaDREB2</i> , <i>TaDREB3</i>	AP2/ERF family transcription factor	Wheat and Barley	[136]
<i>OsNAC45</i>	NAC family transcription factor	Rice	[137]
<i>OsNAC5</i>	NAC family transcription factor	Rice	[138,139]
<i>TaNAC69</i>	NAC family transcription factor	Wheat	[140]
<i>OsZFP245</i>	Protein degradation	Rice	[128]
<i>OsSIK1</i>	Protein degradation	Rice	[141]
<i>OsWRKY11</i>	Protein degradation	Rice	[142]
<i>OsDSG1</i>	Protein degradation	Rice	[143]
<i>OsD1S1</i>	Protein degradation	Rice	[144]
<i>OsD1R1</i>	Protein degradation	Rice	[145]
<i>Os.GH3</i>	Auxin metabolism	Rice	[146]
<i>IPT</i>	Cytokinin Biosynthesis	Rice	[147]
<i>OsbHLH148</i>	Jasmonate signalling	Rice	[148]
<i>OsSKIP1</i>	Stress response	Rice	[149]
<i>OsDHODH1</i>	Pyrimidine nucleotide biosynthesis	Rice	[150]

Table 5: Transgenic developments in cereals for drought stress tolerance.

Gene	Function to counteract stress	Crop	References
<i>AVP1</i>	Osmoregulation and glycine betaine synthesis	Maize	[151]
<i>LOS5</i>	ABA biosynthesis	Rice	[152]
<i>AP37</i>	Transcription factor	Rice	[153]
<i>CBF3</i>	Transcription factor	Rice	[152]
<i>OsNAC10</i>	Transcription factor	Rice	[154]
<i>ZAT10</i>	Transcription factor	Rice	[150,155]
<i>NPK1</i>	Transcription factor	Rice	[152]
<i>Na+/H+ antiporter (NHX1)</i>	Ion transport	Rice	[152]
<i>Ser/Thr Kinase (SOS2)</i>	Ion transport	Rice	[152,156]

Table 6: Transgenic drought tolerant cereal crops tested in field studies.

prevented to a greater extent.

Abiotic Stress Tolerance in GM Cereals Crops

Abiotic stresses especially salinity and drought remains the greatest constraint to crop production and cause for approximately 70% of yield reduction in crops [100]. The significance of transgenics in cereal crops for improving yield under drought stress is apparent from the variety of genes transferred targeting different pathways. These experimental transgenics were tested in controlled environments (Table 5) and in field conditions (Table 6). Genes involved in signaling cascades and in transcription control such as mitogen activated protein (MAP) [101], salt oversensitive kinases [102], phospholipases [103] and transcription factors for instance, Heat Shock Factor (HSF) and the C-Repeat-Binding Factor (CBF) /Dehydration Responsive Element Binding Protein (DREB) and ABA-responsive element binding factor/ABA responsive element (ABF/ABRE) [104], have been extensively studied [105], and used for transgenic plant development against various abiotic stresses in cereal crops.

DREB factors are especially important as these regulate multiple genes involved in drought, salinity and freezing. DREB genes have been used in transformation of cereal crops especially rice and wheat to increase drought tolerance [106,107]. Recently, over expression of *OsDREB2A* significantly enhanced drought and salt tolerance of transgenic rice plants [108] and overexpression of *ZmDREB2A* with CaMV35S or rd28A promoter resulted in better tolerance to drought in maize [109]. NAC is a plant specific Transcription Factor (TF) family with proteins exhibiting a highly conserved N-terminal DNA-binding domain and a diversified C-terminal domain. NAC was derived from the names of first three described proteins containing the DNA binding domain namely, NAM (No Apical Meristem), ATAF1 (*Arabidopsis thaliana* activating factor1) and CUC2 (Cup-Shaped Cotyledon) [110,111]. Transcription factor, *SNAC1* was constitutively expressed in rice and contributed tolerance to severe drought and salt stress during reproductive and vegetative growth without any negative phenotypic effects or yield loss [112]. The WRKY superfamily of plant Transcription Factors (TFs) has a conserved sequence (WRKYGQK) at their N-terminal end [113]. Transgenic rice expressing *OsWRKY11* under control of heat shock protein promoter (*HSP101*) were shown to survive longer and retain water under a short severe drought treatment than wild type plants [114].

Heat Shock Proteins (Hsps) and Chaperones

Heat shock proteins, molecular chaperones and Late Embryogenesis Abundant (LEA) proteins are involved in plant abiotic stresses. Transgenic plants in Basmati rice with overexpression of *HSP101* resulted in significant improvement in plant growth recovery after heat stress [115]. Rice plants transformed with *HVA1* (*Hordeum vulgare* abundant protein) gene encoding group 3 LEA proteins showed dehydration tolerance [116].

Amino Acids

Proline accumulation correlated with improved plant performance under salt stress has been documented in number of studies [117]. In plants, proline biosynthetic pathway from glutamate proceeds through the action of a determining enzyme Δ^1 -Pyroline-5-carboxylate synthetase (*P5CS*). Stress induced increase in proline content is caused by concerted induction of proline biosynthesis genes and repression of proline catabolism genes. Transgenic plants over expressing Δ^1 -Pyroline-5-carboxylate synthetase (*P5CS*) in most of cereal crops including rice, maize and wheat showed salt tolerance.

Amines

Glycinebetaine is most widely studied osmoprotectant and provide tolerance against salinity and drought. Rice plants were transformed with choline dehydrogenase gene (*codA*) from *Arthrobacter globiformis*. The *codA* gene catalyzes the oxidation of choline to glycinebetaine with betaine aldehyde as intermediate product. Transgenic plants with *codA* gene showed recovery after salt stress and seed setting was normal compared to wild type plant [118]. Genetic engineering for increased biosynthesis of polyamines in plants protected them against drought stress. Transgenic rice plants expressing Arginine decarboxylase gene (ADC) from *Datura stramonium* under control of monocot promoter (*Ubi-1*) produced higher level of putrescine under drought stress and protect the plants [119].

Sugar

Trehalose, a non-reducing sugar is present in many bacteria and fungi and in some desiccation tolerant higher plants. Rice tolerance to multiple abiotic stresses through engineering for trehalose overexpression was reported in several studies [120,121]. Transgenic rice plants with increased trehalose level found to be photosynthetically efficient and tolerant to photo-oxidative damage experienced during stress. Mannitol is another sugar alcohol that accumulates upon water and salt stress thus alleviates abiotic stresses. Transgenic wheat expressing mannitol-1-phosphate dehydrogenase gene (*mtl1D*) of *E. coli* was significantly more tolerant to water and salt stress [122].

Recent development in transgenics for abiotic stress was from Monsanto in the year 2012. A transgenic drought tolerant maize variety MON 87460 was developed through *Agrobacterium*-mediated transformation that expresses the cold shock protein B (*CspB*) from *Bacillus subtilis*. The *CspB* protein is an RNA chaperone associated with enhanced abiotic stress tolerance in bacteria and plants, through its interaction with RNA secondary structures, limits their misfolding and maintains cellular functions under various stress conditions. MON 87460 was developed to provide reduced yield loss under conditions in which water was limited as compared to conventional maize [123].

Nutritional Enhancement in GM Cereal Crops

Huge populations in developing countries subsist on a diet of staple crops such as cassava (*Manihot esculenta*), wheat, rice, and maize.

Nutrient	Crop species	Genes	Reference
Vitamin A	Maize (<i>Zea mays</i>)	<i>PactB and Pact1</i>	[175]
	Maize (<i>Zea mays</i>)	<i>Zmpsy1, PactB and Pact1, Glycb</i>	[162]
	Maize (<i>Zea mays</i>)	<i>Zmpsy1, Pact1</i>	[163]
	Wheat (<i>Triticum aestivum</i>)	<i>Zmpsy1, Pact,</i>	[176]
	Rice (<i>Oryza sativa</i>)	<i>NPPSY1, Eucrt1</i>	[159]
	Rice (<i>Oryza sativa</i>)	<i>ZmPPSY1, Eucrt1</i>	[160]
Vitamin C	Corn (<i>Zea mays</i>)	<i>Osdhar</i>	[177]
Folic Acid	Rice (<i>Oryza sativa</i>)	<i>Atgtpch1, Atadcs</i>	[164]
Iron (Fe)	Rice (<i>Oryza sativa</i>)	<i>Osnas2</i>	[173]
	Rice (<i>Oryza sativa</i>)	<i>Gmferritin, Afphytase, Osnas1</i>	[178]
	Rice (<i>Oryza sativa</i>)	<i>Osnas3</i>	[179]
	Corn (<i>Zea mays</i>)	<i>Gmferritin, Afphytase</i>	[180]
Zinc (Zn)	Rice (<i>Oryza sativa</i>)	<i>Onas2</i>	[173]
	Rice (<i>Oryza sativa</i>)	<i>Gmferritin, Afphytase, Osnas1</i>	[178]
	Rice (<i>Oryza sativa</i>)	<i>Osnas3</i>	[179]

Table 7: Transgenic crops with enhanced mineral and vitamin content.

These staple diets are poor sources of macronutrients such as essential amino acids and fatty acids, essential micronutrients such as minerals (iron and zinc) and vitamins (vitamin A). Many transgenic strategies are available to enhance the nutritional value of crops. These strategies were tried in cereal crops and transgenic plants with enhanced level of nutrients obtained in various studies are summarized in Table 7. The chosen approach depends predominantly on whether the nutritional compound is synthesized *de novo* by the plant or obtained from the surrounding environment. The plant synthesizes organic molecules, such as amino acids, fatty acids and vitamins. Increasing the nutritional value requires engineering of metabolites with the aim of increasing the amount of desirable compound, decreasing the amount of a competitive compound or modifying existing metabolic pathway to generate a novel product [157]. By contrast, mineral nutrients are obtained by plant from the environment; therefore, mineral enhancement involves strategies to increase uptake, transport and/or accumulation in harvestable tissues.

Vitamin A

Vitamin A deficiency (VAD) is one of the most prevalent deficiency diseases in developing countries, affecting more than 4 million children each year, up to 500,000 of whom become partially or totally blind [158]. β -Carotene, a precursor of vitamin A does not occur naturally in the endosperm of rice. Transgenic rice first produced with engineered pathway for β -carotene was yellow in color and was given name as Golden rice [159]. Biochemical analysis confirmed that the yellow color of grains represented β -carotene (provitamin A). The level of β -carotene in 1 gram of transformed rice was 1.6 μg , which is sufficient to provide 15-20% of daily need for vitamin A. Scientists at Syngenta [160] developed a new generation of golden rice using an enzyme from maize to dramatically enhance carotenoid levels of grain over 20 times (37 μg carotenoids/g seed). According to an estimate, half of the daily recommended dose of vitamin A upto 3 year old child could be sufficiently met with 72 g of dry golden rice (GR2) [161]. The same genes expressed in maize have yielded kernels containing up to 57.3-60 $\mu\text{g/g}$ dry weight of β -carotene [162,163]. Feeding studies data showed that Golden rice has higher bioavailability of β -carotene and better than spinach at providing vitamin A to children. The basic research for meeting the bioavailability of vitamin A in golden rice got accomplished and future requirement is to conduct field trials and food safety test for further commercial use. The Gates foundation in April 2011 announced US\$10 million grant to International Rice Research Institute, Philippines for the development and food safety tests of golden rice in Philippines and Bangladesh. Field trials for golden rice were carried out in Philippines in January 2013. The golden rice will be available for commercial cultivation in Philippines when all biosafety tests will be satisfied and the level of β -carotene will be sufficient to take care of vitamin A deficiency (www.goldenrice.org).

Vitamin B9

Rice is genetically engineered for vitamin B9 synthesis in grain. Folate (Vitamin B9) is a tripartite molecule combining pterin, p-aminobenzoate (PABA) and one or more glutamate moieties, resulting from three separate metabolic pathways in different subcellular compartments. Genetic modification in rice involved *GCH1* (GTP cyclohydrolase 1), which enhanced the cystolic (pterin) branch and the other *ADCS1* (Arginine decarboxylase), which enhanced the PABA branch in endosperm, resulted in 100-fold increase in folate levels [164].

Vitamin E

Genetic engineering for higher level of vitamin E requires over expression of genes involved in tocochromanol synthesis. This is possible with either increasing the tocochromanol amount or modifying tocochromanol synthesis towards α -tocochromanol, a more utilizable form. Transgenic maize with constitutive expression of two cDNA clones encoding *p*-hydroxyphenylpyruvate dioxygenase (HPPD) and 2-methyl-6-phytylplastoquinol methyltransferase (MPBQ MT) increased the tocopherol content by threefold in endosperm [165].

Essential Amino Acids

Cereals are poor source of lysine and threonine; staple legumes are poor in tryptophan, methionine and/ or cysteine [166]. Two strategies have been devised to deal with amino acid deficiency: (1) engineering plants to produce proteins containing essential amino acids; and (2) engineering amino acid metabolism to increase the availability of essential amino acids in the free amino acid pool. Lysine was the first target in both strategies. Transgenic rice and wheat line were developed expressing lysine rich pea legumin in endosperm and showed 4.2% increase in lysine [167,168]. Successful attempts were also made by expressing lysine-rich animal proteins such as porcine α -lactalbumin in maize lines showing increased lysine content [169]. The other successful instance was engineering of a seed storage protein from *Amaranthus hypochondriacus* (AH) in cereal crops. The AH protein is rich in all the essential amino acids and ideal for humans. Transgenic maize seeds obtained with AH protein has up to 32% more protein than wild-type seeds and packed with higher levels of lysine, tryptophan and isoleucine [170].

Iron Content and Bioavailability

Iron deficiency is the most prevalent form of mineral malnutrition in the developing world with more than 2 billion people at risk of anemia. One of the major challenges with iron is that its mobility in the rhizosphere is dependent on the soil conditions, because only the ferrous form (FeII) is soluble and bioavailable to plants whereas the ferric form (FeIII) is sequestered into insoluble complexes with soil particles [171]. Plants have evolved two counter strategies, one of which is to secrete reductases into the soil to convert ferric iron into the soluble ferrous form, and the other is to release chelating agents known as phytosiderophores (PS) that can be reabsorbed by the roots as PS-Fe³⁺ complexes. Iron levels in plants can therefore, be improved by increasing the export of both reductases and phytosiderophores, for instance, by overexpressing the enzymes Nicotianamine Synthase (NAS) and/or Nicotianamine Aminotransferase (NAAT), which are involved in phytosiderophore synthesis [172]. Transgenic rice plants expressing the *NAS* genes *Osnas1*, *Osnas2* or *Osnas3* accumulated up to 19 $\mu\text{g/g}$ of iron in the endosperm [173]. There was two-fold increase of iron content in rice plants with the expression of ferritin gene from *Phaseolus vulgaris* and iron bioavailability was increased through the introduction of phytase gene from *Aspergillus fumigatus* in endosperm [174]. Transformed rice showed increased phytase level up to 130-fold, giving phytase activity sufficient to completely degrade phytic acid. The nutritionally rich rice with more iron content and better phytase activity has great potential for improving iron requirement in developing countries.

Phytoremediation in GM Cereal Crops

Soils are heavily contaminated with organic pollutants like Polybrominated Diphenyl Ethers (PBDEs), Polychlorobiphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs) and several other pesticides [181]. The remediation of contaminated soils is becoming a challenge all over the world [182]. Phytoremediation is an emerging

Gene Involved	Origin	Crop	Detoxifying capability	Reference
<i>P450CYP</i>	Mammalian cytochrome	<i>Oryza sativa</i>	Enhanced detoxification and tolerance towards various herbicides	[203]
<i>CYP1A1</i>	<i>Homo sapiens</i>	<i>Oryza sativa</i>	Enhanced metabolism of chlorotoluron, norflurazon; Remediation of atrazine and simazine, metolachlor and sulphonylurea	[204-208]
<i>CYP2C19</i>	<i>H. sapiens</i>	<i>Oryza sativa</i>	Phytoremediation of atrazine, metolachlor and sulphonylurea	[206-208]
<i>CYP2C9</i>	<i>H. sapiens</i>	<i>Oryza sativa</i>	Tolerance to sulphonylurea and other herbicides	[208,209]
<i>CYP2B6</i>	<i>H. sapiens</i>	<i>Oryza sativa</i>	Metabolism of ethofumesate and benfuresate; Remediation of metolachlor, atrazine, sulphonylurea and broad range of herbicides	[210,211,205-208]
<i>CYP2B22 CYP2C49</i>	<i>Sus scrofa</i>	<i>Oryza sativa</i>	Tolerance to several herbicides	[212]
<i>Protox</i>	<i>Bacillus subtilis</i>	<i>Oryza sativa</i>	Resistance to diphenyl ether herbicide oxyflufen	[213]
<i>Protox</i>	<i>Myxococcus xanthus</i>	<i>Oryza sativa</i>	Resistance to herbicide and tolerance to oxidative stress	[214]
<i>Mer A</i>	Bacteria	<i>Oryza sativa</i>	Conversion of Hg (II) to Hg (0) in aquatic systems	[199]
<i>arsM</i>	<i>Rhodospseudomonas palustris</i>	<i>Oryza sativa</i>	Arsenic methylation and volatilization	[200]

Table 8: Studies for developing transgenic cereals for phytoremediation.

technology that utilizes plants to clean up organic pollutants and toxic metals in water, sediments, or soils [183-191]. Candidate plants selected for phytoremediation should be resistant to the chemicals or pollutants to be removed. They must be able to metabolize and immobilize the pollutants, because plant and root growth is often inhibited by pollutant-induced stress. Biomass of the selected candidate plant has to be large enough to remediate huge amount of pollutants from the soil [192].

Studies have been reported in plants to detoxify harmful effects of explosives like 2,4,6-Trinitro toluene (TNT), glyceroltrinitrate (GTN) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 2,4,6-trinitrophenol (TNP) [186-192]. The remediation ability of plant can be further improved by genetically modifying plants with genes having potential to degrade pollutants [190]. Most of the work on developing transgenics for phytoremediation has been carried out in dicot plants [193-198]. The remediation ability of plant can be improved by transforming plants with genes having potential to degrade pollutants using transgenic approaches.

Rice appeared as a potential candidate in cereals for phytoremediation of herbicides and toxic metals. Studies in which rice has been modified with different genes having potential to detoxify heavy metals and herbicides are given in Table 8. Transgenic rice with gene *MerA* was found to tolerate and grow in 250 μM of mercuric chloride as compared to wild type plants. This was the first report of a transgenic monocot for phytoremediation of mercury [199]. Recent studies stated that biotransformation of arsenic into organic arsenicals can be done by oxidation, reduction and methylation. The Japonica rice was transformed with *arsM* gene from soil bacterium *Rhodospseudomonas palustris*. This transgenic rice lead to arsenic methylation and volatilization thus can become a potential candidate for phytoremediation of arsenic [200]. Phytoremediation is the prospective approach to detoxify organic pollutants and heavy metals; therefore it is important to find potential genes with better phytoremediation [201]. Gene expression and microarray approaches can be used to identify not only the genes directly involved in herbicide metabolism but also the transcription factors that regulate different mechanisms [202]. Thus, combination of genetic engineering methods and improved phytoremediation will provide the ultimate solution in cleaning up heavily contaminated locations.

Conclusions

The first generation biotech crops realized a significant increase in yield and production by protecting crops from losses caused by pests, weeds, and diseases. These include *Bt* maize, *Bt* rice, glyphosate resistant maize and rice and drought tolerant maize. The second-

generation biotech crops will offer farmers additional new incentives for improving quality of products for example vitamin A enriched rice. The development and release of biotech herbicide tolerant wheat was suspended in year 2005 in North America, however looking at the need of herbicide tolerance in wheat, the work got revived in 2012. Many countries and companies are now fast tracking the development of a range of biotech traits in wheat including drought tolerance, disease resistance and grain quality. Biotech wheat is expected to be ready for commercialization in 2017. In maize, the transgenic drought tolerant MON 87460 was commercially released in North America in 2013. There are speculations for the release of golden rice in Philippines in 2014-2015 and GM maize and rice in China with a potential of benefiting ~1 billion people. The deployment of biotech maize and rice on a wider area as a crop and herbicide tolerance as a trait (First in maize and then in other crops) acted as catalyst for further adoption of transgenic cereals.

However, the increasing cultivation of GM crops has raised a wide range of concern with respect to food safety, environment effects and socioeconomic issues. The major concerns are related to possible toxicity and allergenicity of GM foods and products. The environmental risks include the introgression of transgenes into natural crop populations, impact of gene flow, effect on non-target organisms, evolution of pest resistance and loss of biodiversity. Wider acceptability of GM crops has raised a series of social and ethical issues, which includes restricted access to genetic resources and new technologies, loss of tradition of saving seeds and dominance of private sector and capital investing technology for poor farmers.

The success stories of GM crop cultivation are available in many studies. A study assessing the global economic and environmental impacts of biotech crops for the first fifteen years (1996-2010) of adoption showed that the technology has reduced release of greenhouse gas emissions from agriculture equivalent in removing nine million cars from the roads [215]. The GM crop cultivation has significantly reduced the In the USA, adoption of GM crops resulted in pesticide use reduction of 46.4 million pounds in 2003 [216]. Herbicide tolerant crops have facilitated the continued expansion of conservation tillage, especially no-till cultivation system, in the USA. The adoption of conservation and no-till cultivation practices saved nearly 1 billion tons of soil per year [217]. Cultivation of *Bt* corn in Philippines didn't show any indication that *Bt* corn had negative effect on insect abundance and diversity. The adoption of genetic engineering for cereals will strongly depend on public acceptance of technology and demonstration that genetic modification can result in safe cereal products ultimately providing food for the world's population.

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