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Is genetically modified crop the answer for the next green revolution?

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Abbreviations: 4CL, 4-coumarate CoA ligase; AGP, ADP-glucose pyrophosphorylase; Bt, *Bacillus thuringiensis*; C3H, 4-coumarate 3-hydroxylase; C4H, cinnamate 4-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; CAld5H, coniferaldehyde 5-hydroxylase; CCoA-OMT, S-adenosyl-methionine caffeoyl-CoA/5-hydroxyferuloyl-CoA-O-methyltransferase; COMT, caffeate O-methyltransferase; CRW, corn root worm; ECB, European corn borer; EU, European Union; F5H, ferulate 5-hydroxylase; FHB, fusarium head blight; GM, genetically modified; GMHT, genetically modified herbicide resistant; ha, hectares; H, haemaglutinin; HCT, hydroxycinnamoyl transferase; PDR, pest derived resistance; PRSV, papaya ring spot virus; QPM, quality protein maize; RYMV, rice yellow mottle virus; TSP, total soluble proteins; USA, United States of America; WSMV, wheat streak mosaic virus

Post-green revolution advances made in biotechnology paved the way of cultivating the high-yielding, stress and disease resistant genetically modified (GM) varieties of wheat, rice, maize cotton and several other crops. The recent rapid commercialization of the genetically modified crops in Asia, Americas and Australia indicates the potentiality of this new technology. GM crops give higher yields and are rich in nutritional values containing vitamins and minerals and can thus help to alleviate hunger and malnutrition of the growing population in the under developed and developing countries. It could also be possible to develop more biotic and abiotic stress resistant genotypes in these crops where it was difficult to develop due to the unavailability of genes of resistance in the crossing germplasms. However, further research and investigations are needed to popularize the cultivation of these crops in different parts of the world. This review provides an insight of the impact of GM crops on contemporary agriculture across the past few decades, traces its history across time, highlights new achievements and breakthroughs and discusses the future implication of this powerful technology in the coming few decades.

Introduction

As predicted by population analysts, global population will exceed 9 billion by 2050, when approximately 90% of the global population will reside in developing nations of the poor continents of Asia, Africa and Latin America. Today, 815 million people in these countries are affected by malnutrition, unhygienic living conditions and poverty. During the 1960s and 1970s, high-yielding varieties of rice and wheat developed through conventional genetic manipulation of dwarfing genes brought by the 'Green Revolution' in Asia, were one of the most important accomplishments of the 20th century.[1,2] In the case of wheat and rice, record yields were achieved with the development of semi-dwarf varieties characterized by lodging resistance and nitrogen responsiveness.[1,3,4]

However, it has become very difficult to fulfill the demands of the growing population by developing cultivars using conventional breeding approaches only; due to unavailability of desirable genes in crossable gene pools of a particular and over-exploited crop species. But recent advances in biotechnology has opened new door of transferring the desirable genes from any sources including inter-kingdom gene transfer as the result of the development of transgenic crops. These crops often referred to as genetically modified (GM) crops represent promising technologies that can make a vital contribution to global food, feed, fertilizer, fiber and fuel security. These transgenic crops had numerous advantages over the natural ones. They can avoid food scarcity in the third world countries due to their higher yields,[5] alleviate the deficiencies of protein, minerals and vitamins and can be used to combat malnourishment in impoverished countries[6,7] (e.g., 'Golden Rice', genetically enriched with vitamin A and iron); confer resistance to insect pests through the bioengineering of a specific gene, from a soil-borne bacterium, *Bacillus thuringiensis* (Bt) resulting in the elimination of the pest damage without application of pesticides;[8,9] increase the yield by enhancing tolerance against drought and salinity tolerance.[10-12] Moreover, research is in progress for developing transgenic crops that can be used as edible vaccines, e.g., Khandelwal et al.[13] developed transgenic peanut (*Arachis hypogaea* L.) expressing hemagglutinin

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Table 1. An updated list of important GM/transgenic biotechnology breakthroughs or advances with respect to different GM crops

Year	Advances in plant biotechnology	See references
1930 onwards	Higher yields of wheat, maize, sorghum, pearl millet exploiting hybrid vigour	5
1970	Recombinant DNA technology	65
1977	Discovery of T-DNA transfer into plant cell	66
1983	First transgenic tobacco plants by Agrobacterium mediated transformation	114
1987	First transgenic cotton plants by Agrobacterium mediated transformation	115
1988	First transgenic soybean plants using Agrobacterium-mediated transformation	116
1992	First biolistic generated wheat plants	117
1994	First biolistic transformation in tobacco and maize	118
1998	Papaya with coat protein mediated resistance against *Papaya Ring Spot Virus*	44, 45
1999	ECB resistant Bt corn hybrids	9
1999	Transgenic rice resistant to *Rice Yellow Mottle Virus*	40
2000	Golden rice rich in iron and vitamin A	6, 7
2000	Transgenic potato with resistance to the fungus *Verticillium dahliae*	38
2000	Production of caffeine deficient tea coffee	58
2002	QPM maize containing lysine	93
2003	Enhancing the quality of livestock feed	67
2005	Edible vaccines for measles, cholera, hepatitis B are being developed in India	55, 56
2005	Genetically modified plants yielding high biomass	63, 114
2007	Commercial release of transgenic herbicide-tolerant rice in Costa Rica	119
2009	Development and Regulation of Bt Brinjal	120

(H) protein of rinderpest virus. Oral immunization of mice with transgenic peanut induced H-specific antibodies indicates potential for producing an edible vaccine for rinderpest. Herman et al.[14] used the transgene-induced gene silencing approach to shut down the gene that codes for the protein believed to cause most soybean allergies. During the last two decades, a number of breakthroughs occurred in transgenic biotechnology with respect to GM crops and has been listed in **Table 1**, and the major components and output of GM technology platform has been schematically presented in **Figure 1**.

In spite of the above mentioned benefits of transgenic crops over the conventionally bred cultivars, their production remained restricted; and it was not until the 1990s that these transgenic crops were introduced on an experimental basis commercially (**Fig. 2**).[1,3] Unfortunately, this relatively new technology is facing resistance from different sectors. Attempts have been made to create unjustified fear about the potential adverse impacts of GM foods/products or plants and crops on human health and the environment[2,15-17] to the extent that GM experimental materials are being destroyed.[18] But it is important to note that in the US more than 60% of all processed foods contain transgenic ingredients, but not a single transgenic food product has yet been clearly shown to have any harmful effect(s) based on any acceptable well defined animal model trial.[19] In contrary to the popular belief the transgenic crops expressing *cry* genes do not pose a threat to mammalian health.[20] Although there is a possibility of gene flow to wild species through a misplaced transgene exists in some cases,[21-23] in most cases it is unlikely to happen because of the difficulty of hybridization between a transgenic crop plant and its wild relatives and the need for embryo rescue to obtain a hybrid under laboratory conditions.[17] Hence, during the past few years, fast adoption of transgenic crops has been seen on the rise as indicated by increased acreage of area under transgenic crop cultivation (**Fig. 2**). This reflects the significant advantage of transgenic crops appreciated by some farmers in industrial and developing countries leading to certain extent of commercialization of transgenic crops.[24]

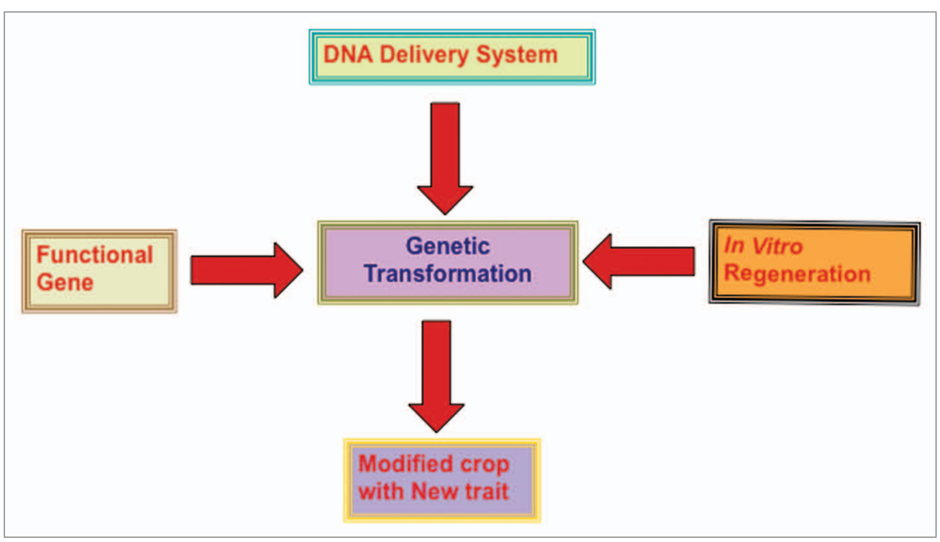

Figure 1. Essential components and output of the GM technology platform.

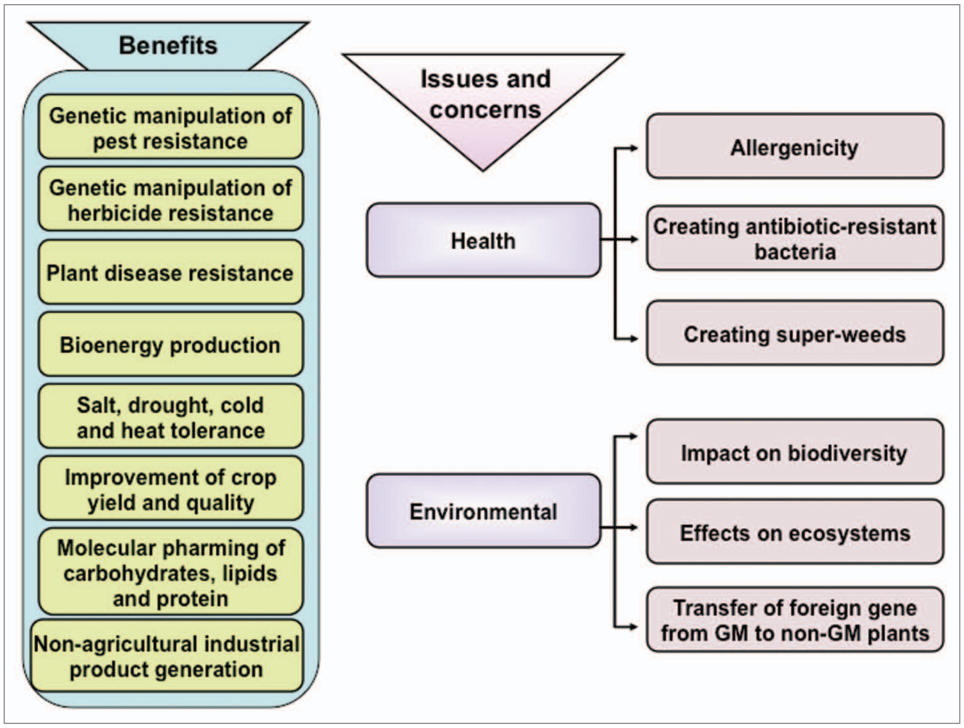

Figure 2. Pros and cons of the GM technology.

Since, GM technology is an important weapon in the war against global poverty and starvation, crop improvement through genetic engineering has in fact become a reality.[17,25-27] Although the United States of America (USA) have commercialized the practice of cultivating GM crops, European Union (EU) still restricts this technology for being adapted for rapid commercialization in agricultural sector. There is no true and clear evidence to suggest that GM foods directly pose any threat to human and animal food and feed safety; however, further researches have to be done and there is an urgent need of education and reassurance regarding the potential benefits of GM crops.[19,20]

Alleviating the Production and Quality Constraints Through the GM Crops

During the past years, GM cultivars have been developed in different crops. Till 1995, before the cultivation of GM crops for commercial production, a number of transgenic varieties have been developed for different crops. These developments led to initiation of GM crops cultivation in 1996 at a commercial scale (**Table 2**). A list of different transgenic (GM) crop varieties that are either released for research field trials or grown at commercial scales across the globe have been summarized in **Table 3**. These crops particularly focused on improvement of productivity and quality of current cultivars. In following sections, we have reviewed the progress made in commercialization of GM crops against abiotic and biotic stresses as well as for improving the quality in order to address the malnutrition problems prevalent especially in the third world countries.

Biotic Stresses

The purpose behind crop protection is not elimination of pests but to reduce crop losses to an economically acceptable level.[28] Hence, novel crops modified to express durable disease resistance should present farmers with the opportunity to reduce herbicide, fungicide and insecticide applications. Insect pests cause tremendous losses to yield all over the globe. For example, European Corn Borer [ECB, *Ostrinia nubilalis* (Hubner)], causes a loss of up to 2,000 million dollars annually in the USA alone.[29] This loss was attempted to be minimized through bioengineering of a gene from a soil bacterium, *B. thuringiensis* (Bt), that confers almost complete resistance to ECB. This resulted in development of high yielding Bt-corn hybrids to fight the infestation by ECB.[29] Another most devastating pest of corn is the corn root worm (CRW) in the United States.[30] In the past, options for controlling the western corn rootworm included crop rotation and the application of insecticides. Later development of GM corn showed potential for resistant against CRW. In 2003, first GM corn was approved in USA, which showed resistance against the CRW.[30] Recently, EU approved the import of the GM maize MIR604 from USA, developed by Syngenta that produces an effective Bt protein to combat the CRW. However, CRW resistant GM corn cultivation application is still pending with the EU.

The Bt cotton is an important example illustrating the fact that the transgenic crops are now increasingly grown at commercial scale in Asian countries like China[31] and India.[32] One of the studies also showed that Bt rice has the potential to reduce yield loss caused by Lepidopteran insects,[33] which is estimated from

Table 2. Development of transgenic varieties in different crops for commercial release

Crop	Trait	Varieties	Year of release	Releasing organizations/ country
Insects/Pest Resistance				
Cotton (*Gossypium hirsutum* L.)	Bt-(Cotton Bollworm, tobacco bud worm and pink bollworm)	Bollgard	1995	Monsanto
	Bt gene	NA	NA	Calgene
Potato (*Solanum tuberosum* L.)	*Bt* gene (Colorado potato beetle)	New Leaf	1995	Monsanto
Maize (*Zea mays* L.)	*Bt* gene (Corn borer)	Maximizer	1995	Northrup King (Sandoz)
	Bt gene (Corn borer)	Yieldgard	1996	Monsanto
	Bt gene (Corn borer)	NA	NA	Northrup King
				Novartis
				Mycogen
	Corn Root Worm	MIR604	2003	Monsanto
Squash (*Curcubita pepo* L.)	Virus resistance	Freedom	NA	Poineer Hi Bred
				Asgrow Seeds
Tobacco (*Nicotiana tabacum* L.)	Virus resistance	NA	NA	China
Tomato (*Lycopersicon esculentum* (L) Karsten)	Virus resistance	NA	NA	China
Capsicum (*Capsicum annuum* L.)	Virus resistance	NA	NA	China
Cantaloupe (*Cucumis melo* L. subsp. *melo* var. cantalupensis Naudin)	Virus resistance	NA	NA	Asgrow Seeds
Herbicide Resistance				
Maize (*Zea mays* L.)	Glufosinate	Libertylink	1996	AgrEvo
	Sethoxydim	SR	NA	BASF
	Glufosinate	NA	1996	Dekalb Genetics
	Glyphosate	Roundup Ready	NA	Monsanto
	Glufosinate	NA	NA	Plant Genetics Systems
Conola (*Brassica napus* var. *napus* L.)	Glufosinate	Inovator	NA	AgrEvo
	Glyphosate	Roundup Ready	NA.	Monsanto
	Bromoxynil	NA	NA	Rhone-poulenc
Cotton (*Gossypium hirsutum* L.)	Bromoxynil	BXN	1995	Calgene
	Sulfonyl urea	NA	1996	Du Pont
	Glyphosate	Roundup Ready	1996	Monsanto
Soybean (*Glycine max* (L.) Merr.)	Glyphosate	Roundup Ready	1995	Monsanto
Flax *(Linum usitatissimum L.)*	Sulfonyl urea	Triffid	NA.	Canada
Quality				
Tomato (*Lycopersicon esculentum* (L) Karsten)	Vine ripened shelf life (Antisense RNA Technology)	FlavrSavr	1994	Calgene
	Vine ripened shelf life (Antisense RNA Technology)	Endless & Summer	1995	DNA Plant Technology
	Delayed Softening	NA	1995	Zeneca
	Thick skin paste consistency			
	Delayed ripening	NA	1995	Monsanto
Potato (*Solanum tuberosum* L.)	Modified starch	NA	NA	AVEBE
Conola (*Brassica napus* var. *napus* L.)	Oli characteristics (High lauric acid)	Laurical	1995	Calgene
	Male sterility hybrid system			AgroEvo

Source: Kumar et al.;[121] NA, Information not available.

Table 3. List of currently reported GM lines, cultivars, varieties and traits including experimental field release or trials as well as for commercial cultivation

Name of the crop	Line name/ Commercial variety/Trait name	Year of release	Country	Area under cultivation (hectares)	Company name	Special property/gene	References[§]
Rice (*Oryza sativa* L.)	Phosphoinothricin (Bar) Rice	2000	California, China, Japan, Philippines, Indonesia, Vietnam	20,000	AgrEvo	Phosphinothricin herbicide resistance bar	6, 7, 107
Wheat (*Triticum aestivum* L.)	NA	1996	Canada, Germany, Australia, USA	46,000	Monsanto	Cytogenetic manipulations by removing the pairing regulator *Ph1* resulted in increased pest tolerance, higher yields	122, 123
Maize (*Zea mays* L.)	MON 810, Agrisure GT (GA21)	1996/97	France, Spain, Germany, North America, U.K., Canada	60,000	Monsanto, Mahyco	*CEPSPS* gene (glyphosate herbicide resistance) from Agrobacterium C4, tolerance to glyphosate-based herbicides	107
Cotton (*Gossypium hirsutum* L.)	Bt cotton	1995	United States, Argentina, Canada, China, India Mexico, Brazil, Kenya	12.1 million	Monsanto, Mahyco, Syngenta	A *cry1A* gene stacked with *cry2A* gene of *B. thuringiensis* resistant to Lepidopteran insects	99, 107
Potato (*Solanum tuberosum* L.)	Alpha, Norteña, Rosita	1996/99	Netherlands, Mexico, Germany, Mediterranean countries	0.1 million	Bayer	*cry3A* gene, putative viral helicase and replicase genes, imparts resistance to Colorado potato beetle and potato leafroll virus	124
Tomato (*Lycopersicon esculentum* (L) Karsten)	Flavr Savr, Ohio 8245, Proago PGs	1994	Argentina, Brazil, China, Mexico	NA*	Calgene	Increased shelf life (reduced ethylene)-Truncated ACC synthase gene, delayed ripening (reduced ethylene)-SAM hydrolase, resistance to Lepidopteran insects-*cry1Ac* gene	107
Papaya (*Carica papaya* L.)	SunUp, UH Rainbow	1998	USA, New Zealand	0.1 million	NA	Viral coat protein gene resistance to papaya ringspot virus	124
Oilseed rape (*Brassica napus* L.)	GT73	1996	Canada, France, Japan, USA, UK	2.7 million hectares	Monsanto, AgrEvo	*EPSPS* gene from Agrobacterium C4 and *gox*-glyphosate herbicide resistance, thioesterase gene-high laurate and myristic acid seed content, *bxn*-oxynil herbicide resistance	101
Squash (*Curcubita pepo* L.)	NA	2005	USA, Hawaii	0.1 million	NA	Viral coat protein gene-Resistance to watermelon mosaic virus 2, zucchini yellow mosaic virus, cucumber mosaic virus, watermelon mosaic virus 2, zucchini yellow mosaic virus	107
Soybean (*Glycine max* (L.) Merr.)	NA	1996/97	USA, Argentina, Canada, Mexico, Romania, Uruguay, South Africa	33.3 million	Monsanto	*EPSPS* gene from Agrobacterium C4 which imparts glyphosate herbicide resistance	99, 124
Tobacco (*Nicotiana tabacum* L.)	Bxn tobacco	1996	France, China	NA	NA	Oxynil herbicide resistance-*bxn*	107
Sugar beat (*Beta vulgaris* L.)	RR® sugar beet	2008	USA, Canada	257,975	Monsanto	Herbicide resistance	53

[§]Source: AGBIOS data base. Available online at: http://www.agbios.com; [*]NA: Information not available.

2 to 10% of Asia's annual rice yield.[34] Field trials of transgenic rice suggested high tolerance against yellow stem borer.[35] In China, insect-resistant GM variety 'Xianyou 63' developed by inserting the Bt-gene;[36] showed resistance to other insect pests such as rice stem borer [*Scirpophaga incertulas* (Walker)] and leaf roller [*Cnaphalocrocis exigua* (Butler)].

It has also been shown that engineering with anti-fungal genes could help produce crop plants resistant to fungal pathogens;[37,38] expression of antifungal peptide defensin in transgenic potato (*Solanum tuberosum* L.) conferred resistance to the fungus *Verticillium dahliae* Klebahn. In another work, Tu et al.[39] introduced the cloned *Xa21* gene (a gene with broad spectrum resistance to bacterial blight of rice caused by the bacteria *Xanthomonas oryzae*) into a widely grown rice variety IR72 with promising yield attributes and better disease resistance.[41] A transgenic approach based on Pest Derived Resistance (PDR) was successfully employed to produce the Rice Yellow Mottle Virus (RYMV)-resistant rice variety.[41]

Transgenic wheat plants, engineered with the coat protein gene of Wheat Streak Mosaic Virus (WSMV) conferred protection against some WSMV strains.[42] The PDR technology offers a promising means for inducing virus resistance in a variety of plants[43] including potato.[44] Coat-protein-mediated resistance has helped to control Papaya Ring Spot Virus (PRSV) in papaya (*Carica papaya* L.) in Hawaii.[45,46] Genetically Modified Herbicide Resistant (GMHT)-associated herbicides (glyphosate and glufosinate) are less persistent than conventional herbicides, such as atrazine,[47] which is now prohibited across the EU,[48] and control of certain broad-leaf and grass weeds can be achieved with a single herbicide.[49] However, the new weed species that are poorly or not controlled by glyphosate is emerging very fast all over the world.[50,51] The new transgenic crops with additional herbicide-resistance genes will be the only solution to deal with this problem in near future.

Abiotic Stresses

For abiotic stresses, efforts were made to develop transgenic crops against different stresses. Abebe et al.[10] demonstrated that wheat engineered with the *mtlD* gene from *Escherichia coli* had improved tolerance to water stress and salinity. A year earlier, Garg et al.[52] showed that overexpression of *E. coli* trehalose biosynthetic genes (*otsA* and *otsB*) as a fusion gene provided increased tolerance to abiotic stress in rice, resulting in elevated capacity for photosynthesis under drought and low-temperature stress conditions. We can see that transgenic/GM cultivar and varieties are currently being grown at commercial scales across the globe for different crops (**Tables 2 and 3**). Although, these efforts could not help to commercialize GM crops by providing protection against abiotic stresses, it is expected to control abiotic stresses through commercialization of drought tolerance varieties of maize by 2012, or earlier in the USA and by 2017 in Sub-Saharan Africa where maize is the staple food.[53]

Nutritional Enhancement

Biofortification. By engineering rice with two genes from daffodil and two from the bacterium *Erwinia uredovora*, Potrykus and his collaborators designed rice strain to produce pro-vitamin A. Later, by incorporating the iron-synthesizing ability in it, they were able to produce rice grains rich in pro-vitamin A as well as iron.[6,7] The resulting rice, called "Golden Rice" is rich in nutritive value. Ducreux et al.[54] reported the development of high carotenoid potato tubers with enhanced concentrations of β-carotene and lutein through the application of metabolic engineering. A novel transgenic approach involving organ-specific gene silencing on tomato used to increase the content of both carotenoids and flavonoids, which are highly beneficial for human health.[55]

Edible vaccines. Transgenic approach is a useful way to provide food crops for immunization against deadly diseases like hepatitis or tuberculosis. These edible vaccines are much safer because they can be administered without refrigeration, hypodermic syringes or needles. Using such a novel vaccination approach, we can save millions of people who simply die due to lack of access to traditional inoculants.[56] In this connection, transgenic peanut (*Arachis hypogaea* L.) expressing hemagglutinin (H) protein of rinderpest virus has been developed.[13] The experimental results obtained after oral immunization of mice with transgenic peanut showed induction of H-specific antibodies indicating the potentiality of producing an edible vaccine for rinderpest. In another very interesting work, Charles Arntzen, of the Biodesign Institute at Arizona State University, has genetically engineered potatoes to produce a vaccine against hepatitis B virus. Vaccines against pneumonia and bubonic plague orally immunogenic to mice have also been recently developed.[57] The efforts are also made in India to produce the edible vaccines against measles, cholera and hepatitis B.[58,59] Although quite inspiring, it is important to mention that these are emergent research areas and technology platforms and will take considerably long time before they are actually available for commercial release and production.

Bioenergy or Biofuel Crops

Bioenergy/biomass energy is now playing an essential role to reduce fossil-based transportation fuels as a viable alternative, and to reduce CO_2 emissions for environmental and economic sustainability. Biofuel obtained from cellulosic plant matter, supplemented by grain ethanol, has been predicted to decrease the need for petroleum fuel in the future.[60,61] But, currently, the commercial production of cellulosic ethanol has been facing two main constrains: the high costs of production of cellulases in microbial bioreactors, and the costs of pretreating lignocellulosic matter to break it down into intermediates and remove the lignin to allow the conversion of cellulases to biomass cellulose.[61]

As an answer to the problem, genetically engineered plants are being considered to produce cellulases and hemicellulases within the crop biomass rather than producing cellulases in fermentation tanks. Plant genetic engineering has already been used successfully to produce different groups of cellulases (endoglucanases, exoglucanases and β-glucosidases) in plants.[62] The heterologous enzymes can also be extracted from fresh or dry transgenic crop biomass as part of the plant total soluble protein (TSP), which can then be added to pretreated crop biomass for conversion into fermentable sugars.[63,64]

In a second approach, plant genetic engineering technology offers great potential to modify lignin amount and/or configuration in order to reduce the needs for expensive pretreatment processes.[65] Ragauskas et al.[66] indicated that downregulation of lignin biosynthesis pathway enzymes to modify the chemical structures of lignin components and/or reduce plant lignin content is an important potential way to reduce pretreatment costs in bioethanol production from cellulosic biomass. Downregulation of 4-coumarate 3-hydroxylase (C3H) in alfalfa resulted in a shift in the lignin profile and subsequently altered lignin structure causing improved digestibility.[65,67] In another study, downregulation of cinnamyl alcohol dehydrogenase (CAD) in alfalfa resulted in modification of lignin residue composition, while in Populus spp., CAD downregulation resulted in improved lignin solubility in an alkaline medium, highlighting more efficient delignification and the possibility of decreasing the need for pretreatment processes in this species.[68] Shifting energy from lignin biosynthesis to polysaccharide synthesis has been achieved in aspen (*Populus tremuloides*). Downregulation of 4-coumarate CoA ligase (4CL) resulted in a 45% decrease in lignin content and a concomitant 15% increase in cellulose content in this species.[69] Surprisingly, a 52% reduction in lignin content and a 30% increase in cellulose content were reported when coniferaldehyde 5-hydroxylase (CAld5H) was also downregulated.[70] Importantly, a recent study showed that downregulation of six different lignin biosynthetic pathway enzymes in alfalfa, namely cinnamate 4-hydroxylase (C4H), hydroxycinnamoyl transferase (HCT), C3H, S-adenosyl-methionine caffeoyl-CoA/5-hydroxyferuloyl-CoA-O-methyltransferase (CCoA-OMT), ferulate 5-hydroxylase (F5H), and caffeate O-methyltransferase (COMT), could reduce or eliminate the needs for chemical pretreatment in the production of fermentable sugars.[71,72] Some of these transgenic alfalfa plants produced nearly twice as much sugar from cell walls as do wild-type plants upon conversion.

By genetically modifying plants modified for plant growth regulators, increased amount of overall feedstock biomass could also be achieved. Eriksson et al.[73] reported transgenic hybrid poplar with increased gibberelin biosynthesis displayed improved growth and an increase in biomass, probably due to the effects of gibberelin on plant height. In another study, genetic manipulation for higher expression of ADP-glucose pyrophosphorylase (AGP), a key enzyme for starch biosynthesis in endosperm, has produced promising results attaining 20% increase in plant biomass in rice.[74] Metabolic engineering is also playing an important role in improving biofuel and sugar production. In a recent study it is reported that induced expression of a yeast glycerol-3-phosphate dehydrogenase has led to an increase in lipid production in oil-seed rape.[75]. Another recent study indicated that the overexpression of a bacterial origin sucrose isomerase in vacuoles could double the sugar yield in sugarcane.[76]

Currently, there is growing interest in utilizing algae as a potential source of biofuel including starches for alcohols, lipids for biodiesel and hydrogen for fuel cells. [77] Increasing cellulose solubility can increase saccharification, therefore providing another potential route to decreasing pretreatment needs.[65] Transgenic expression of levansucrose from the bacterium *Erwinia amylovora* (which mediates the synthesis of water-soluble fructan from sucrose) increases permeability of algal cell walls.[78] Graves et al.[79] and Kawasaki et al.[80] reported algae expressing exogenous hyaluronan and chitin synthase in the extracellular matrix elevated cellulose production. Considerable progress in identifying relevant bioenergy genes and pathways in algae and significant breakthroughs in the development of powerful genetic engineering tools will certainly provide further advances in algal-based biofuels in very near future.

Increasing crop biomass by genetic engineering is another promising area of research. Delaying the onset of flowering has been reported to increase biomass,[81,82] and improving architectural features such as dwarf stature and erect leaves could potentially increase biomass and yield.[83] The various possibilities that genetic engineering can offer to increase bioethanol production from these crops include modifying biomass properties to reduce processing costs or increase biomass yield and reducing agricultural inputs.[84] The quality of livestock feed has also been enhanced. This feed is currently derived from imported GM soybean and GM maize products (corn gluten feed and distillers dried grain). Increasing the ruminant digestible material of straw by 20% would upgrade its nutritional value to that of hay.[85]

The future for the large-scale production of cellulases within the crop biomass and the subsequent promising replacement of microbial reactors with plants as biofactories seem brighter with the boom of biotechnology. With the success of current ongoing researches, it is comprehended that plant genetic engineering is able to produce low cost transgenic biofuel resources and bring those to market for a better biofuel economy by deconstructing plant cell-wall polysaccharides, suppressing enzymes for lignin biosynthesis and/or increasing the yield of plant biomass. Since transgenic biofuel crops would be used in an industrial platform and will not be a part of food or feed chain; the resistance or negative feelings as is prevalent for GM crops are not expected against plain transgenic fuel crops of the future. Although transgenic biofuel crops are not a part of food and feed chain, bioconfinement of transgenes should be considered in constructing transgenic energy crops to prevent transgene flow by out-crossing with wild relatives.

Other Advantages of Transgenic Plants and their Products

Crops modified with an increased level of citrate exudation have a higher capacity to use insoluble forms of phosphate, yielding higher biomass when grown in phosphate-limiting conditions.[86] Engineering Irish-specific potato crops with a similar trait could reduce the use of phosphate fertilizers which leads to eutrophication. Kato et al.[87] cloned the gene encoding caffeine synthase from young leaves of tea, paving the way for creating tea and coffee plants that are naturally deficient in caffeine, thus meeting the consumer demand of decaffeinated coffee. Decaffeinated coffee is now being grown on genetically modified coffee bushes that could yield low-caffeine beans in 3 or 4 years.[88,89] Scientists in England successfully introduced pentaerythritol tetranitrate reductase, the bacterial enzyme that initiate degradation of explosive residues, into plants, and the transgenic plants so created were used for bioremediation of contaminated soils.[90,91] Genetic engineering can also be used to reduce food borne allergies. Herman et al.[14] used the transgene induced gene silencing to shut down the gene that codes for the protein believed to cause most soybean allergies.

Development of GM Crops with Stacked Traits

Progress has also been made in development of GM crops carrying improved feature for two or more traits or stacked traits. Such GM crop meets the multiple needs of farmers and consumers. Till date 11 countries including the USA, Canada, Philippines, Australia, Mexico, South Africa, Honduras, Chile, Colombia, Bolivia and Argentina, (7 of the 11 are developing countries) are growing these crops and it is expected that more countries will adopt GM crops with stacked traits in the future. In 2008, a total of 13.3 million hector was planted under stacked biotech crops compared with 10.5 million hectares in 2007.[92] In the USA, the fastest growing component of stacked maize was the triple stacks conferring resistance to two insect pests plus herbicide tolerance. Double stacks with pest resistance and herbicide tolerance in maize were also the fastest growing component in 2008. In Philippines the doubling of biotech maize was 25% in 2007 to 57% in 2008. Biotech maize with eight genes, named Smartstax™, is expected to be released in the USA in 2010 with eight different genes coding for several pest resistant and herbicide tolerant traits. Future stacked crop products will comprise both agronomic input traits for pest resistance, tolerance to herbicides and drought plus output traits such as high omega-3 oil in soybean or enhanced pro-Vitamin A in Golden Rice.[53]

Role of Public and Private Sectors in Production of Transgenic Crops

(a) Commercialization of transgenic crops. Iron deficiency, a most common dietary deficiency among the poor nations, affects especially children and women of reproductive age. In pregnant women, severe anemia may cause fetal growth retardation and large-scale maternal deaths.[93] Nearly 400 million people in the world are reported to be at risk of vitamin A deficiency, leads to blindness and premature death.[94,95] Golden rice developed by transgenic technology is rich in iron and vitamin A which can alleviate malnutrition and avert blindness among millions of children, and is therefore referred to as the "grains of hope."[6,7] Nutritional values of food crops were also increased for example Quality Protein Maize (QPM).[96,97] The transgenic cotton producing insecticidal proteins commonly referred to as Bt cotton, is now widely grown in many countries.[98] The Bt cotton with a single *cry1A* gene and stacked also with *cry2A* gene has provided satisfactory protection against the damage by the Lepidopteran bollworms, especially the cotton bollworm [*Helicoverpa armigera* (Hubner)].[99] The baseline susceptibility of the larvae of *H. armigera* to *Cry1Ac* and other toxins carried out in many countries has provided a basis for monitoring resistance. There is no evidence of development of field-level resistance in *H. armigera* leading to the failure of Bt cotton crop anywhere in the world, despite the fact that Bt cotton was grown on the largest ever area of 12.1 million hectares in 2006 and its cumulative cultivation over the last 11 years has surpassed the annual cotton area in the world. Bt cotton is providing millions of farmers with increased yields, reduced insecticide costs and fewer health risks.[98]

(b) Impact of GM crops on agriculture growth in developed and developing countries. *(1) In developed countries.* GM crops such as cotton, maize and soybeans have been planted widely by farmers since 1995 in countries like the USA, Canada, Brazil, Argentina and China.[98] Depending on the country or the geographic region(s) concerned; the acceptance or rejection of GM crops correlated closely with the presence or absence of regulatory directives from the respective governments. While in some parts it was accepted with great enthusiasm and in other parts with an air of skepticism. However, government regulation and policies had a strong influence on the acceptance/rejection pattern globally. The planting of the GM crops have not spread significantly beyond the countries mentioned above and represent around 98% of the GM crop acreage currently.[98]

Conscious policy choice has now become a second reason for the restricted spread of GM crops. While some governments like the US and Canada have taken a well established regulatory agencies and policies toward new GM crop technologies, other governments have taken a more cautious view. The countries led the way in screening GM crop technologies for food safety and bio safety risks using essentially the same methods employed for conventional crops; then allowing private markets for GM crops to operate without any new labeling or segregation restrictions. Governments in EU and Japan were more cautious since strong oppositions grew among domestic consumers, environmental organizations and anti-globalization advocacy groups, in these geographical areas over time.[100]

(2) In developing countries. Unlike the USA and the EU, the public sector has developed many useful transgenic crops in countries like Argentina, Brazil, India, Kenya and China. Transgenic crops are not being commercialized because of intellectual property and regulatory constraints that in the first three of these countries regulatory authorities have not yet given farmers official permission to plant any GM crops; and in China, where farmers have been permitted to plant GM cotton, regulators are still holding back on the release of most GM food and feed crops, even though China's own national agricultural research system has invested a considerable effort in developing such crops. In China, most of the Bt cotton varieties and all of the transgenic tobacco and tomato lines commercialized were developed by public research institutions.[101] The results of a two-year survey of smallholders in Makhathini Flats, KwaZulu-Natal (South Africa) show that farmers who adopted Bt cotton in 1999–2000 benefited according to all the measures used. Higher yields and lower chemical costs outweighed higher seed costs, giving higher gross margins.[102]

In March 2002, the Indian Government permitted commercial cultivation of Bt cotton, and the country now has 3 years of experience with the crop.[98] In 2002, some 38,000 ha were planted with Bt cotton, with over 12,000 ha of this being in the state of Maharashtra grown by over 17,000 farmers. All of the Bt cotton in India has been developed and released by Monsanto (the owner of the Bt gene) in partnership with a local seed company (Mahyco) based in Mumbai. As of 2004 Monsanto is working with a further three local companies to develop Bt cotton varieties for different regions of India. None of these companies sell seed directly to farmers, but instead work through a large network of dealers. The dealers may or may not offer credit to cotton growers. The situation has been further complicated with the advent of 'illegal' Bt cotton produced locally in the country. The GM industry claims, based on trial data, that while these varieties may be cheaper they do not perform as good as the 'legal' varieties.[98]

Although officially recognized for having increased production and farmers' income, Bt cotton, remains highly controversial in India. Among other allegations, it is accused of being the main reason for a resurgence of farmer suicides in India. However in specific regions and years, where Bt cotton may have indirectly contributed to farmer indebtedness (via crop failure), leading to suicides, its failure was mainly the result of the context or environment in which it was introduced or planted; Bt cotton as a technology is not to blame. Mosanto claims destruction of crops has been caused by Spodoptera against which Bollgard in Bt cotton is ineffective. Overall results suggest that while the cost of cotton seed was much higher for farmers growing Bt cotton relative to those growing non-Bt cotton, the costs of bollworm spray were much lower. While Bt plots had greater costs (seed plus insecticide) than non-Bt plots, the yields and revenue from Bt plots were much higher than those of non-Bt plots (some 39% and 63% higher in 2002 and 2003, respectively). Overall, the gross margins of Bt plots were some 43% (2002) and 73% (2003) higher than those of non-Bt plots. Thus on an average it can be said Bt cotton production benefited the farmers. The outcomes of GM crops in India were quite comparable with countries like China, Mexico, Brazil and Kenya.[98]

(c) **Trends of production and productivity of GM crops.** A total of 13.3 million hectares of GM crops have been planted by the farmers in 2008, this was the 10% of total 140 million hectares. Crop wise account of GM production was also high for soybean (51%), corn (31%), cotton (13%) and canola (5%). The number of countries joining the team of GM producers has reached a historical count of 25 in 2008.[53] More than half of the total area 62.5% of GM crops was planted in USA and rank first in the GM crop production. Argentina with 21% was second and Brazil with 15.8% was third.[98] Canada also grows genetically modified crops like canola, maize, soybean and sugar beat on 7.6 million hectares. India is another largest GM crop producer with 7.6 million hectares in 2008, mainly for cotton.[98] China was the first country to grow a commercial GM crop transgenic tobacco in 1992. China had 3.8 million hectares land used for GM crops. Paraguay and South Africa also have 2.7 and 1.8 million hectares land under GM crop cultivation in 2008. Egypt planted Bt maize for the first time in 2008 thereby becoming the first country in the Arab world to commercialize biotech crops.[103] Mainly two types of GM crops were grown all over the world (1) Herbicide tolerance (63%) and (2) Insect resistance (18%) rest is for Quality and Yield related. For these traits, different crops like soybean, maize, cotton and rapeseed are the major GM crops being grown from genetically engineered seed. Squash, papaya, potato, poplar and some vegetables are the other GM crops that are grown over relatively lesser acreages. Recently most popular area biofuel production from the crops have highlighted the use of GM crops for biofuel production, including 7 million hectares of corn used in ethanol and 4 million hectares of soybean used in biodiesel. However, there is no commercially available GM crop designed for biofuels. Despite the significant adoption over relatively limited time period, the global acceptance scenario shows that the level of adoption of GM technology varies quite drastically over geographic, social and political demographies.

Summary and Future Directions

The major breakthroughs in food productivity that saved lives particularly in Asia, was started the Green Revolution in the late 1960s; came from conventional plant breeding approaches.[104] However, the enormous used of pesticides, fertilizers, mechanization and irrigation have introduced serious economic, social and environmental challenges. The development of the GMO or the Gene Revolution is an attempt to solve the problems associated with the Green Revolution. Advances in genetic engineering and biotechnology have made rapid strides since 1983 when the first transgenic plants were produced. Currently reliable and efficient transformation protocols are available for a variety of plants, which include cereals, grain legumes, forage crops, oilseed crops, fiber crops, ornamentals and forest trees. Genetic transformation offers direct access to an unlimited pool of desirable genes; not previously accessible to breeders, particularly in case of inter or intra kingdom gene transfers. The successful deployment of transgenic approaches to combat insect pests and diseases of important crops like rice, wheat, maize barley and cotton, is a remarkable accomplishment pest-resistant genetically modified crops can and are contributing to increased yields and agricultural growth in many developing countries and benefiting small-scale farmers.[24]

The multi-million dollar losses from insect pests suffered by cotton farmers have been reduced by the use of Bt cotton.[105] Biofortification of crops to reduce or alleviate malnutrition among the poor masses constitutes another exciting development. Countries like India have already initiated programs to breed the nutritional qualities into popular rice varieties.[106,107] Vasconcelos et al.[108] have shown that the soybean ferritin gene driven by an endosperm specific glutelin promoter resulted in high accumulation of iron and zinc levels in brown rice as well as in polished transgenic grains. Such a nutritional enhancement of a cereal crop would be unimaginable by conventional means alone. Transgenic technology also finds application in the production of edible vaccines for immunization against deadly diseases like hepatitis B or tuberculosis, two of the serious diseases of the poor masses in

Africa and Asia. Gene technology is believed to have the potential to increase productivity, reduce the incidence of famine and malnutrition in developing country. It has been promoted as a green technology to protect biological diversity.

As with any other new technology, genetic engineering is not without adversaries, some of which even go as far as destroying experimental materials. This anti-science zealotry[2] and public hostility to modern biotechnology has been attributed to "lack of scientific literacy"[109] and may impede human progress. The opponents of the new technology believe that plant biotechnology is unnatural, unsafe, and inherently wrong, and that it results in harmful products. Though till date there is no solid evidence to suggest that GM foods pose any threat to human safety, however work needs to be done on informing and reassuring the public about the global benefits of GM crops. Mass awareness is essential to realize the full potential of these boons of modern technology. In developing countries the governments should provide education and adequate funding to the farmers in order to encourage the production of GM crops. It is important to realize the full potential of these boons of modern technology can bring 'green to gene' and 'gene to evergreen' revolution in this century.

The impact of scientific crop breeding became more prominent after the 1960s and resulting varieties were utilized judiciously to protect local populations from mass starvation that challenges the developing and under developed countries of the world till date.[110] The green revolution was able to put out a number of wheat, rice and maize varieties in a short span of time on their ability to utilise water, sunlight and plant nutrients effectively. The most significant aspect of the success of the green revolution has been the use of novel genotypes specifically adapted for local growing conditions. However, this revolution was confined to assure irrigation areas and unsustainable use of agrochemicals that enabled direct yield increases. It has also been said that new plant varieties displaced landraces in the fields and thus led to a loss of biodiversity. Whereas, evergreen revolution is appropriate blend of different approaches to sustainable agriculture such as plant biotechnology, organic farming, green agriculture and eco-agriculture.[111,112] The genetic improvement of food crops and the science of plant breeding are changing rapidly to meet the needs of the 8.3 billion people in 2025.[110] Genetic engineering and conventional breeding methodologies have the potential for solving agricultural problems where traditional techniques have failed.

The father of green revolution and Nobel laureate Norman E. Borlaug coined the term gene revolution and he defended the use of genetically modified crops could offer numerous new possibilities in future. This gene revolution, propelled by genetic engineering, allows different combinations of traits to achieve yield increase, reduced agricultural chemicals, reduced vulnerability to the whims of nature and improved nutritional content.[113] New technologies for developing GM crops are still very costly and the biotech industries need to gather the necessary funds to develop these technologies. The private industries typically focus on their markets for generating their profit and still GM crops are largely the product of private biotech industry. But, it may be too early to predict the varying adoption rates and benefits of gene revolution technologies for farmers. If the green revolution brought so many benefits, the evergreen revolution would bring all those benefits in perpetuity, taking advantage of the gene revolution.

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