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Genetic Engineering of Field, Industrial and Pharmaceutical Crops

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Abstract

Ability to modify plants at the genomic level by advanced molecular technology has enhanced the scope of improvements in plant traits attempted earlier through conventional breeding methods. Techniques such as genetic transformation have opened new vistas whereby functional genes, not commonly present in a particular species can be added from other species. The traits incorporated into the genetically engineered plants in the beginning were confined to those governed by dominant genes, e.g. insecticide resistance and herbicide tolerance but advancements with time now also permit the transfer of complexly inherited traits such as drought and cold tolerance. Transgenic technology is also useful in understanding gene expression and metabolic pathways which can then be used to harness the full genomic potential of the plant. This review presents a narrative on development of transgenics and their use for the improvement of field, industrial and pharmaceuticals crops. In addition, discussions are made on current status on genetically modified crops, hurdles to genetic engineering, overcoming strategies and future scope.

Keywords

Genetic Engineering (GE), Transgenic, Genetically Modified (GM), Field Crops, Industrial Crops, Pharmaceutical Crops, Molecular Farming

1. Introduction

The alteration or modification in an organism's genome using modern DNA technology is called genetic engineering or genetic modification. Since it involves the introduction of foreign DNA or synthetic genes into the organism of interest the resulting artifact is often referred as transgenic and or genetically modified (GM). The ability to introduce alien genes from distant species or life forms into plants has made available an entirely new and novel gene resource pool to breeders in their pursuit to improve crops for survival, productivity, and products. Transgenic crops can be generated with the use of recombinant DNA techniques which alter the crop's ge-

netic makeup by manipulating the genome—either by introduction, deletion, substitution, or silencing of an individual gene or group of genes of interest. The functionality of transgenes has expanded with time. In the beginning, only traits that exhibited complete dominance, free of the interaction from the native plant genome or the environment, were targeted. For such traits, only one copy of the trait introduced into one of the inbred parents was required. Fortunately, some of these dominant traits such as insect resistance and herbicide tolerance provided solution to major production hurdles encountered by farmers in producing major crops of food and fiber.

Cultural practice modification offered by early transgenic varieties not only enhanced economic return to the farmer because of higher crop yields but also had multiplier effects of soil erosion prevention from reduced tillage, and reduction in environmental pollution from the residual herbicides and insecticides. Now, the scope of transgenic technology has expanded to include quantitative traits such as stress tolerance and yield improvement that necessitate the interaction of the introgressed genes with native genes engaged in the metabolic pathway for the phenotypic trait expression where environment may also influence considerably the final phenotypic expression. Stacking of introgressed genes in hybrids to create value combination of traits is also receiving considerable attention. Genetic engineering (GE) is usually resorted when improvement through conventional breeding and mutagenesis have been exhausted. Such situations arise if a desired trait is not present in the crop germplasm, the trait has proven difficult or very time consuming to improve through conventional breeding, or there is a need to remove or switch off particular genes. Commercialization of first genetically engineered crop started back in 1996 and since then it has reached new heights in its application and wide adaptability to various sectors of modern agriculture. Since 1996 to 2013 there has been tremendous increase in the acreage of genetically engineered crops. Between 1996 and 2013 there has been more than 100 fold increase in the acreage of genetically engineered crops [1].

2. Development of Transgenic

The ability of genetic engineering to incorporate foreign traits into plants was first exhibited in 1970s. Although approved earlier for limited sale, it was not until 1996 that the Monsanto Company (USA) got the approval to commercially market European corn borer (*Ostrinia nubilalis*) resistant corn (*Zea mays* L.), Colorado potato beetle (*Leptinotarsa decemlineata*) resistant potato (*Solanum tuberosum* L.), cotton bollworm complex (tobacco budworm—*Heliothis virescence*, bollworm—*Helicoverpa zea*, and pink bollworm—*Pectinophora gossypiell*) resistant cotton (*Gossypium hirsutum* L.), and a non-selective, broad spectrum herbicide glyphosate (N-phosphonomethylglycine) tolerant soybean (*Glycine max* (L.) Merr.). The genes for all insecticidal proteins, modified *cry*1*Ab* in corn [2], modified *cry*1*Ac* in cotton [3], and modified *cry*3*Ab* in potato [4] derived from commonly found soil bacteria—*Bacillus thuringiensis* (*Bt*). Glyphosate tolerant soybean was developed by incorporating a bacterium glyphosate resistant EPSP (5-enolpyruvyl shikimate-3-phosphate) synthase gene [5]. The transgenic crop research developed over years can be grouped into three generations (**Figure 1**). Each generation represents unique thrust areas for developing transgenic crops and has contributed to the present pool of commercially adopted transgenic crops [6].

Modern agriculture has been quick to adopt the commercial first generation transgenic crops expressing herbicide tolerance and insect resistance since these effects were clearly visible in the crop production systems. The second generation transgenic crops were designed for product quality characteristics but they did not lived up to expectations, and no commercial crop with these specific characteristics is presently in the market. However, the first approved transgenic food, Calgene's Flavr Savr tomato which reached the market in 1994, succeeded in delaying softening of the ripe fruit after harvesting but was a complete commercial failure and was withdrawn from the market in 1997 out of safety concerns. The third generation of transgenic crops has been engineered for use as biofactories or living reactors in the production of pharmaceuticals and industrial chemicals and is often referred as "molecular farming".

3. Genetic Engineering of Crops

The early and most cost-reward producing use of GE has been in the development of insecticide and pesticide resistance in field crops. A great deal of interest has currently been shown in incorporating tolerance to environmental stresses in crop cultivars in order to stabilize the yield under fluctuating environmental conditions. In
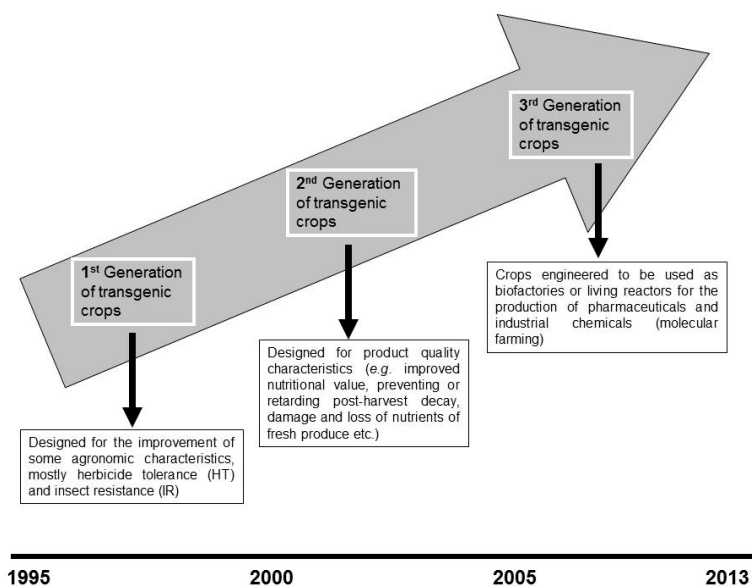
Figure 1. Development and characterization of genetically engineered crops.

addition, as enhanced nutritive value of crop has gathered much interest to combat malnutrition in developing countries and to meet the food preference of naturalists, several transgenic cultivars with fortified nutritive values have been released. Some degree of success has also been accomplished in developing crops with chemical constituent of industrial value and the use of plants as hosts for pharmaceutical products.

3.1. Field Crops

Similar to the wide acceptance of hybrid corn due to its yield advantage more than sixty years ago, farmers in the United States now commonly plant transgenic cultivars of several field crops possessing traits that increase their value or yield potential. The typical transgenic traits approved for US crops are herbicide tolerance (bromoxynil, glufosinate, glyphosate, sulfonylurea), insect resistance (Bt kurstaki, Bt tenebrionis), virus resistance (Papaya ringspot virus, cucumber mosaic virus , zucchini yellow mosaic virus, watermelon mosaic virus, potato leaf roll virus, potato virus Y), male sterility (barnase/barstar), modified ripening (ACC synthase, ACC deaminase, SAM hydrolase, polygalacturonase), and modified oils (high lauric , myristic, oleic acids) [7]. Currently, transgenic acreage in several countries (4 - 17 different countries) for soybean, cotton, maize and canola account for 79%, 70%, 32% and 24% respectively of the total planting [1]. **Table 1** lists different transgenic cultivars have been released or are at different stages of development.

The transgenes currently employed in weed management confer resistance to crops allowing use of effective broad-spectrum herbicides. The gene characterizing bacterial enzyme conferring tolerance to glyphosate has been most widely used, however, transgenes conferring tolerance to bromoxynil (Buctril), glufosinate (Liberty), and sulfonylurea (Glean) are also registered [7]. Two classes of *Cry* genes from *Bacillus thuringiensis* have been incorporated to produce *Cry* protein in a number of plants species to control a lipidopteran and co-lipidopteran insects [7]. The protein breaks down in the insect intestine releasing a lethal toxin called delta-endotoxin. Duke [85] enumerated three strategies for alleviating the need for herbicidal kill utilizing transgenes, namely, 1) transgenic alteration of biocontrol agents to make them more effective in managing weeds, 2) transgenes that produce a more competitive crop or provide the ability to produce natural phytotoxins (allelochemicals) and 3) cover crops that will self-destruct near the time of main crop planting alleviating the need for herbicidal kill. The disease resistances of commercial value have been incorporated into papaya (*Carica papaya* L.) to bestow resistance to papaya-ringspot-virus [86] and into rice (*Oryza sativa* L.) to give bacterial leaf blight resistance [87].

Most of phosphorus (P) in the soil is present in insoluble form limiting its availability to the plant. However, López *et al.* [88] observed that engineering to overproduce citrate enhanced the ability of tobacco (*Nicotiana tabacum* L.) plants to use insoluble P in the soil with potential for commercial utilization. Researchers are attempting to improve photosynthesis in C_3 plants with the aim of boosting yield by achieving overexpression of

C$_4$-cycle enzymes [89]-[91] but much work remains to accomplish this goal. In a recent review [92], the potential of transgenic approach in improving rice production has been shown by identifying a range of traits that can be addressed to develop transgenic for commercial adoption and cultivation. Response of a plant to environmental stress can range from altered gene expression and cellular metabolism to changes in crop yields [93]. Genes have been introduced into cereals to fortify the grain nutritional value, e.g., cereals fortified with vitamin A to prevent partial or full blindness and with iron to relieve anemia due to iron deficiency in pregnant woman and children. Golden rice (*Oryza sativa* L.) is the result of the introduction of two genes from daffodils (*Narcissus pseudonarcissus* L.) and one from a microorganism resulting in increased production of vitamin A precursor beta-carotene [94]. Also in rice, genes involved in the production of an iron-binding protein and enzyme that facilitates its availability have been introduced to develop genotypes exhibiting elevated levels of iron [95]. Considerable amount of research has been devoted to enhance nutritive value of different crops with varied success. Researchers have also attempted to incorporate improved quality traits (delayed ripening, enhanced appearance, extended functionality, enhanced sweetness, and fats and oils) and nutritive value (fats and oils, protein, carbohydrate, caroteniod and vitamin E) into crops (**Table 1**).

Table 1. Selected examples of transgenic crops targeting a specific trait or quality for crop improvement.

Scientific name	Common name	Trait/quality	Target gene product/gene/source	Reference
Beta vulgaris	Sugar beet	Herbicide tolerance (glyphosate)	5-Enolypyruvylshikimate-3-phosphate synthase (EPSPS), CP4 strain of *Agrobacterium tumefaciens*	[8]
Glycine max L.	Soybean	Herbicide tolerance (glufosinate ammonium) Carotenoids and vitamin E Fats and oil Protein Heat shock protein	Phosphinothricin acetyltransferase (PAT), *Streptomyces viridochromogenes* Alpha-tocopherol Omega-3-fatty acid; increased oleic acid Methionine enriched glycinin P5CR-Increased proline accumulation	[9] [10] [11] [12] [13] [14]
Gossypium hirsutum L.	Cotton	Insect-resistance Increased ethanol production	cry1F gene, from *Bacillus thuringiensis* var *Aizawa* ADH (Alcohol dehydrogenase)	[15] [16]
Helianthus annuus	Sunflower	Herbicide tolerance (imidazolinone) Fats and oil	By selection of a naturally occurring mutant Docosahaexaenoic	[17] [18]
Lens culinaris	Lentil	Chemically induced seed mutagenesis	Acetohydroxyacid synthase (AHAS)	[19]
Linum usitatissimum L.	Flax, Linseed	Herbicide tolerance (sulfonylurea)	Acetolactate synthase (ALS)	[20]
Brassica napus	Argentine Canola	Herbicide tolerance (glufosinate ammonium)	Phosphinothricin acetyltransferase (PAT), *S. viridochromogenes*	[21]
Medicago sativa	Alfalfa	Herbicide tolerance (glyphosate) (lucerne) Drought and freezing	5-Enolypyruvylshikimate-3-phosphate synthase (EPSPS), CP4 strain of *Agrobacterium tumefaciens* Sod, Mn-Sod, Mn superoxidase dismutase	[22] [23] [24] [25]
Oryza sativa	Rice	Herbicide tolerance (imidazolinone, imazethapyr) Protein Salinity and drought Submergence tolerance Cold tolerance Metal toxicity tolerance	Acetolactate synthase (ALS), ethyl methanesulfonate (EMS) Beta-phaseolin HVA 1 pdc1, Sub1 Wx-control amylase synthesis, Gs2-chloroplastic glutamine synthetase Parβ-Glutathione S-transferase	[26] [27] [28] [29] [30] [31] [32] [33]
Triticum aestivum	Wheat	Herbicide tolerance (imidazolinone) Glyphosate tolerance Enhanced functionality Drought tolerance	Acetohydroxyacid synthase (AHAS), ALS 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS), *Agrobacterium tumefaciens*, strain CP4 Protein modification of high molecular weight glutenins, flour functionality DREB1A, HVA1	[34] [35] [36] [37] [38] [39] [40] [41] [42]

Continued

Zea mays L.	Maize	Insect-resistance Herbicide tolerance (glyphosate) Corn rootworm resistance Drought tolerance	*cry1Ab*, *Bacillus thuringiensis* subsp. kurstaki, modified 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) modified *cry*3A gene, *E. coli* NF-YB, MAPK	[43] [44] [45] [46] [47]
	Sugarcane	Insect resistance	*Cry*1a(*b*), *synthetic cry*1*Ac*, cry1*Aa*3, cry1*Ab*	[48] [49] [50] [51] [52] [53]
Lycopersicon esculentum	Tomato	Delayed ripening Freezing tolerance	1-Aminocyclopropane-1-carboxyllic acid (ACC) synthase afa3	[54] [55]
Cucurbita pepo	Squash	Cucumber mosiac virus (CMV), zucchini yellows mosaic (ZYMV) and watermelon mosaic virus (WMV)	Coat protein (CP)	[56]
Solanum tuberosum L.	Potato	Colorado potato beetle resistance Carbohydrate Enhanced sweetness Freezing Heat shock protein	*cry*3A, *Bacillus thuringiensis* (subsp. Tenebrionis) Amylose and amylopectin structure/ratio Monellin BetA DcHSP17.7-improved cellular membrane stability and enhanced *in-vitro* tuberization	[57] [58] [59] [60] [61]
	Cassava	Carbohydrate	Amylose and amylopectin structure/ratio	[62]
	Sweet potato	Protein	Essential amino acid rich protein	[63]
	Carrot	Salt tolerance (up to 400 mM)	Badh	[64]
	Cucumber	Enhanced sweetness	Thaumatin	[65]
Carica papaya	Papaya	Papaya ringspot virus (PRSV) resistant	Coat protein (CP)	[66]
Cucumis melo	Melon	Reduced accumulation of S-adenosylmethionine (SAM), and consequently reduced ethylene synthesis,	S-Adenosylmethionine hydrolase	[67]
Musa acuminata	Banana	Carbohydrate	Amylose and amylopectin structure/ratio	[68]
Prunus domestica	Plum	Plum pox virus (PPV) resistance	Coat protein (CP) gene	[69]
Nicotiana tabacum L.	Tobacco	Herbicide tolerance (bromoxynil, ioxynil) Reduced nicotine content Salinity Drought Chilling Waterlogging tolerance Metal toxicity tolerance	Nitrilase, *Klebsiella pneumoniae* Tobacco quinolinic acid phosphoribosyltransferase (QTPase) BetA, Mltd p5cs, TPS1, SacB ad7, Des9 ACC MsFer-Ferritin (ion storage); arsC	[70] [71] [72] [73] [55] [74] [75] [76] [77] [78] [79] [80]
Cichorium intybus	Chicory	Male sterility	Barnase ribonuclease, *Bacillus amyloliquefaciens*	[81]
Coffea arabica	Coffee	Resistance to pests (coffee leaf miner, coffee berry borer and nematodes)	*Bt*	[82] [83] [84]

3.2. Industrial Crops

The crop grown as a feedstock in the production of a commodity rather than for direct human consumption is referred to as industrial crop. The industrial crops contribute to the farm income and provide economic stimulus

to rural areas. The products from industrial crops also provide a pool of substitutes for classic imports from other nations. Some examples of industrial crops are: oil palm, rape seed, soybean, safflower, peas, plantago, potato, fiber hemp, flax, guar, agave, cassava, jojoba, kenaf. Fiber crops are amongst the most common industrial crops. Some transgenic plant lines, which produce compounds for specific industrial applications, are already commercialized, and many more are at different stages of development by biotechnology companies, research institutes and universities. Progress has been slow in developing transgenic plants for important industrial uses. However, immense potential exists in employing GE for producing crops for biofuel, pulp and paper, plastics, oil and lubricants, and soil remediation.

3.2.1. Biofuel, Paper and Plastics

Lignocellulosic biomass constitutes an important category of feedstock whose conversion into biofuel is accretive to the environment due to its high carbon sequestration credential. However, there is a major impediment to the process of conversion of the biomass cellulose to ethanol as it is embedded within the lignin of the plant cell walls and not easily separable for direct chemical reaction. This recalcitrance adds extra and cumbersome steps to the conversion process and the release of all cell wall cellulose for utilization remains problematic. Researchers have genetically engineered the switchgrass (*Panicum virgatum*) to address the recalcitrance and reduced ethanol yield [96]. Overexpression of PvMYB4, a general transcriptional repressor of the phenylpropanoid/lignin biosynthesis pathway, has shown promising results by increasing cellulosic ethanol yield from switchgrass by 2.6-fold and a dramatic reduction of recalcitrance [96]. Genetically engineered PvMYB4-OX switchgrass can thus provide a novel system for further understanding cell wall recalcitrance. Similar studies were done on transgenic alfalfa lines where it was found that recalcitrance to both acid pretreatment and enzymatic digestion is directly proportional to lignin content. Modifying the lignin biosynthetic enzymes yielded nearly twice as much sugar from cell walls as wild-type plants [97]. Lignin modification, by genetic engineering, could bypass the need for acid pretreatment and thereby facilitate bioprocess consolidation. Moreover, induction of genes that accelerates lignin degradation can be valuable for pulp and paper production from lignocellulosic biomass as well. Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) enzyme present in white rot fungi has attracted much attention because of its ability to degrade lignin [98]. Hood *et al.* [99] generated transgenic maize plants by employing an *Agrobacterium*-mediated system with fungal laccase gene (EMBL accession no. U44430) which showed highest expression in the maize embryo-preferred globulin 1 promoter and targeting of the protein to the cell wall. High oil germplasm was used to increase germination, as well as to assist in increasing expression 20-fold in five generations through breeding and selection. It was hypothesized that the high oil lines might provide substrate (*i.e.* oil) for the laccase-generated free radicals to act upon, thereby preventing the accumulation of free radicals that alter seed physiology, such as increased lignification. Downregulation of one of the major enzymes involved in lignin biosynthesis, 4-coumarate: coenzyme A ligase (*Pt*4CL1) in transgenic aspen (*Populus tremuloldes*), resulted in a 45% decrease in lignin with a compensation of 15% increase in cellulose, doubling the plant cellulose:lignin ratio without any change in lignin composition and without any apparent harm to plant growth, development or structural integrity [100]. Changing levels of cinnamyl alcohol dehydrogenase (CAD) have also been found to modify lignin synthesis [101]. Poplar (*Populus tremula* × *Populus alba*) with CAD antisense constructs grows similar to control trees but with an increase in the proportion of free phenolic groups in lignin facilitating solubilization and fragmentation [102].

Poly 3-hydroxyalkanoates (PHAs) are a class of microbially produced polyesters comprising of at least 100 different PHA constituents and at least five different dedicated PHA biosynthetic pathways [103] with potential application as biodegradable plastics. Arai *et al.* [104] was able to transfer from *Aeromonas caviae* FA440 modified PHA synthase gene (phaCAc) into *Arabidopsis thaliana* that enabled the plant to accumulate PHA in its tissues.

3.2.2. Industrially Desirable Fatty Acids

Cahoon *et al.* [105] reported that expression of a gene from pot marigold encoding an enzyme that introduces conjugated double bonds into polyunsaturated fatty acids resulted in the accumulation of calendic acid, a novel conjugated polyunsaturated fatty acid, to amounts of 20% - 25% of the reported total soybean seed oil. Calendic acid is even more oxidatively unstable than linolenic acid, thus improving the drying properties of coating applications. However, the level of calendic acid concentration in soybean at 20% - 25% remains much lower than 55% concentration found in the marigold.

Castor (*Ricinus communis* L.) oil contains high levels (up to 90%) of ricinoleic acid needed for conversion to substitutes for petroleum derived lubricants, emulsifiers, inks, and nylons. Unfortunately, castor cultivation is prohibited in most countries as the seeds also contain toxin ricin. The level of ricinoleic acid achieved in tobacco and *Arabidopsis* have been only to the amount of <1% and 17%, respectively [97] [98] [106] [107]. Singh *et al.* [108] observed that although a single gene (FAH12) may regulate ricinoleic acid synthesis, its accumulation in triglycerol most likely required involvement of other genes. Napier [109] noted that, thus far, it has been difficult to attain levels of industrially desirable fatty acids in transgenic plants similar to found in the non-agronomic source plants.

McKeon [110] reported the ongoing efforts to enhance industrial chemical constituents in important crops. Canola with high laurate for detergent and soybean with high oleate for food and monomers have reached commercial stage, while canola with petroselenate for food and monomers, soybean with vernolate for plasticizers and coatings, cotton with low-saturates for food uses are in development.

3.2.3. Phytoremediation

There are many reports of transgenic plants exhibiting tolerance to varying levels of heavy metals, a trait useful for phytoremediation of contaminated soils [111]-[118]. *Arabidopsis thaliana* transformed by type 2 MT (metallothioneins) gene (tyMT) from cattail (*Typha latifolia*) exhibited an increased tolerance to Cu^{2+} and Cd^{2+} [119]. Indian mustard (*Brassica juncea*) plants overexpressing ATP sulphurylase were shown to have higher shoot Se concentrations and enhanced Se tolerance compared to wild type when grown in the presence of selenite [120] [121]. Family of sulfur rich peptides termed phytochelatins (PCs) are able to bind to Cd and some other heavy metals [122] and transgenic tobacco plants over expressing cysteine synthase in either the cytosol or chloroplasts were more tolerant to metals such as Cd, Se and Ni [123]. Transgenic plants have been developed with altered transporter genes with the aim to exclude a toxic metal ion, transporting the metal into the apoplastic space and vacuole where metal would be less likely to exert a toxic effect [124]. Phytoremediation uses different plant processes and mechanisms normally involved in the accumulation, complexation, volatilization, and degradation of organic and inorganic pollutants [125]. **Table 2** shows categorization of different processes used by some of the model transgenic plants in phytoremediation. Most of the information available today is either from laboratory and or greenhouse experiments. Elaborated field testing is required to validate and establish the effectiveness of these transgenic plants for actual cleanup of contaminated metal sites.

3.3. Pharmaceutical Crops

Genetic transformation studies have shown the potential of producing recombinant proteins, including pharmaceuticals and industrial proteins, and other secondary metabolites in plants. Several substances have already been produced in transgenic plants and are in different stages of clinical trials (**Table 3**) but none of them were approved as pharmaceutical for humans until 2012. Recently, an enzyme, for treating the rare hereditary Gaucher disease (Elelyso, developed by Protalix) generated in carrot tissue became the first Plant Made Pharmaceutical (PMP) for human use to gain regulatory approval by the US FDA [140]. At present, many crops are being developed to produce drugs or biologics for the diagnosis, treatment, or prevention of diseases in human and animals. These include enzymes, hormones, anticoagulant factors, vaccines, and monoclonal antibodies targeted at a variety of disease, such as cystic fibrosis or non-Hodgkin's lymphoma [141].

Current therapeutic proteins biosynthesis systems utilizing prokaryotes, yeasts and cultured mammalian cell mediums are handicapped in several ways [142]. Prokaryotes can only biosynthesize simple therapeutic proteins such as insulin, interferon or growth hormone as complex proteins produced in them are not always properly folded or processed for proper level of biological activity [143]. Complex proteins produced from yeasts have co- and post-translational modifications (PTMs) problems while cultured mammalian cells are difficult to scale up to large volume productions due to lack of bioreactor capacities, high operating cost, and are subject to virus or prion contamination [138]. Recent studies have suggested transgenic plants could be suitable alternatives for large scale, low cost, and safe production of complex therapeutic mammalian proteins [144]. A range of plant/crop species have been used in production of commercial pharma/crop products. An extensive review by Saklani and Kutty [145] describes the status on plant derived compounds in clinical trials with a special emphasis on plant-based anticancer drugs.

Using plants as bioreactor can substantially alleviate capacity problem especially in cases of therapeutic

Table 2. Selected example of transgenic plants overexpressing genes for improved phytoextraction and phytovolatilization efficiency and phytodegradation potential.

Target plant	Gene	Product/source	Performance	Reference
Phytoextraction efficiency				
Tobacco, rapeseed	MT2	Metallothionein—human	Enhanced Cd tolerance	[126]
Tobacco, cauliflower	MT1	Metallothionein—mouse	Tolerated 200 mM CdCl$_2$ Tolerated 400 mM CdCl$_2$ in hydroponic medium	[127] [128]
Arabidopsis	PsMTA	Metallothionein—pea	8× higher Cu accumulation	[129]
Indian mustard	gshl	γ-Glutamyl-cysteín (*E. coli*)	3 - 5× higher γ-ECS and GSH levels and 90% higher shoot Cd concentrations	[130]
Poplar	gshI	γ-Glu-cys synthetase (*E. coli*)	Cd tolerance and increase of total sulfur in shoot	[131]
Phytovolatilization efficiency				
Arabidopsis	merB	Organomecurial lyase-bacteria	Volatilization of up to 763 ng Hg (0) min^{-1}·g^{-1}	[132]
Yellow poplar	merApe9	Hg(II) reductase-mutagenized merA	Volatilization of 10× more mercury than WT plants	[133]
Indian mustard	SMT	Selenocysteine methyltransferase *A. bisulcatus*	Volatilized 2.5× more Se than WT plants when supplied with selenate	[134]
Phytodegdration potential				
Tobacco	Onr	PETN reductase	Enhanced detoxification of nitroglycerin	[135]
Poplar	gshl	γ-ECS	Elevated herbicide tolerance and rapid herbicide degradation	[136]
Indian mustard	gshI and gshII	γ-ECS and GS	Enhanced tolerance and 2 - 12× increase in nonprotein thiol level	[137]
Rice, Potato	P450CYP	Mammalian cytochrome	Enhanced detoxification and cross tolerance toward several herbicides	[138] [139]

Table 3. Plant made pharmaceuticals for human use at different stages of regulatory approval.

Product	Crop	Class	Application	Status	Organization	Reference
Glucocerebrosidase ELELYSO/UPLYSO	Carrot cell culture	Therapeutic enzyme	Gaucher's disease	US FDA approved, May, 2012	Protalix	[165]
Alpha-galactosidase (PRX-102)	Carrot cell culture	Therapeutic enzyme	Fabry's disease	Phase I/II	Protalix	[166]
Acetylcholesterase (PRX-105)	Carrot cell culture	Therapeutic enzyme	Biodefense	Phase I	Protalix	[167]
Apo-A1Milano	Safflower	Therapeutic protein	Cardiovascular disease	Preclinical	SemiBioSys Genetics	[168]
Human serum albumin	Flax	Therapeutic protein	Maintenance of blood plasma pressure	Preclinical	Agragen	[169]

antibodies production where rapid volume increases are of paramount concern. Plants also provide a wide array of species suited for use as bioreactors. A recombinant protein yield of up to 20 kg/ha have been reported using tobacco, corn, soybean or alfalfa [146] [147]. Newly developed plant expression systems in duckweed, moss, algae, and higher plant suspension-cultured cells offer the opportunity of fast turnover and high yield molecular farming in highly contained and completely controlled environment [142]. Unlike yeasts, transgenic plants possess the ability to carry out most PTMs needed to produce complex proteins such as plasma proteins, antigens, hormones, cytokines, enzymes and antibodies [144] [148]. In most cases, several PTMs including proteolytic cleavage(s), oligomerization and glycosylation are required to obtain therapeutic proteins of proper biological activity, pharmacokinetics, stability, and solubility [142]. Expression levels for therapeutic proteins in plants remain low and currently several approaches including codon optimization [149]-[151], RNA silencing [152] [153], targeted secretion of recombinant proteins by the roots [154] [155], stored into seed endosperm [156] and seed oilbodies [157], production in chloroplast [158] are being experimented to improve yields. Difference in

the protein molecule *N*-glycans produced by plants from mammalian glycoproteins may also trigger immune responses in humans and induce their fast clearance from the bloodstream [159] [160]. Strategies to obtain plant-derived antibodies with human-compatible carbohydrate profiles include their retention in the endoplasmic reticulum [161] [162] or alternatively transformation of plants with mammalian glycosyltransferase [163] [164]. As a result of these advances, plants have emerged as a potential safe and cost-effective alternative to microbial or mammalian expression systems especially for large quantity multimeric recombinant proteins.

4. Current Status of Genetically Engineered Crops

In the United States, the Animal and Plant Health Inspection Service (APHIS) regulates the development and release of transgenic crops. APHIS classifies phenotypic traits under AP (agronomic properties), BR (bacterial resistance), FR (fungal resistance), HT (herbicide tolerance), IR (insect resistance), MG (marker gene), NR (nematode resistance), OO (others), PQ (product quality), and VR (virus resistance) categories. APHIS issued a total of 2192 permits for different phenotypic traits in 2013. Other traits (OO), herbicide tolerance (HT) and agronomic properties (AP) topped the category by contributing 34.53, 21.94 and 20.12 percent [170] respectively (**Figure 2**). Permit for other traits also topped in year 2014 with more than 75% of total permit issued. One permit can contain multiple phenotypes of phenotype categories while each phenotype category may include one to several traits. The FAO data [171] on developing countries showed more than thousand different GMOs under various stages of commercialization (**Table 4**).

Since 1996 the increase in acreage of transgenic crops in industrial countries has moderated but the pace of planting these crops in developing countries has accelerated (**Figure 3**). The total area planted in genetically engineered crops has increased from 1.7 million hectares in 1996 to 175 million hectares in 2013 reflecting a remarkable increase global hectarage of genetically engineered crops by 100 folds. In 2013, transgenic crops were grown in a total of 27 countries where the top ten countries each grew more than 1 million hectares. A record 18 million farmers, in 27 countries, have planted 175 million hectares (432 million acres) in 2013 [1]. If a trend like this continues, it is expected that 40 or more countries will adopt genetically engineered crops by 2015, the final year of the second decade of commercialization.
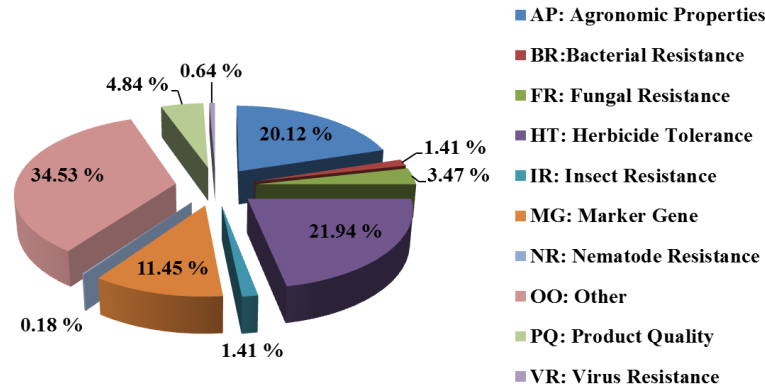

Figure 2. Total permits issued by phenotype for the transgenic crops in the United States during year 2013.

Table 4. Number of genetically modified organisms (GMOs) in developing countries under different stages of development.

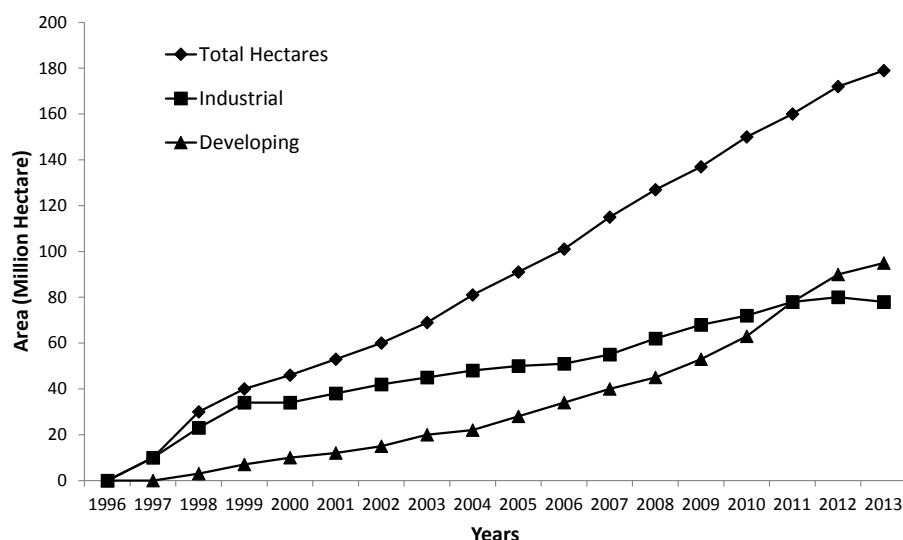| Region/(GMOs)	Experimental phase	Field trial	Commercial phase	Not specified
Asia (679)	453	119	33	74
Africa (85)	39	36	10	-
Latin America and Caribbean (306)	99	185	15	7
Europe (28)	18	6	2	2
Near East (51)	31	16	2	2
Grand total 1149	640	362	62	85

Figure 3. Trend of global growth in acreage of genetically engineered crop production between developing and industrial countries (adapted from Reference [1]).

5. Hurdles to Genetic Engineering and Overcoming Strategies

The rapid commercialization of transgenic technology is not without controversy. In addition to public suspicion or outright opposition, the main scientific concerns regarding deployment of transgenic crops include potential harmful effects to non-target organisms, gene flow into related wild species and persistence of gene products in the environment.

Transgenic technology is still in inception and faces a number of challenges. To start with, current methods of gene insertion are not precise to control the location and number of copies of the gene inserted. As the location has considerable influence on gene expression, if not placed suitably, "silencing" of the inserted gene can occur or inserted gene can silence the native genes. Transgenic traits are not always stable over generation and such instability could be deleterious to fertility, yield or other parameters of plant fitness or usefulness. Oftentimes, the inserted DNAs contain multiple stacked genes with the potential to cause undesirable interactions in the host plant. Pleiotropy is a common form of gene expression. Thus a gene known for a trait deemed desirable may produce unintended harmful side effects. King *et al.* [172] noted that glyphosate applied for weed control in soybean decreased biomass and seed yields when soil moisture was limiting.

There is fear that year-after year production of *Bt* crops will produce pest population resistant to *cry* proteins. This apprehension is appropriate as it has happened in the past after an insecticide was applied repetitively. The only way to overcome the threat from the acquired resistance by insects is to infuse into the plant new defense mechanism or build multiple barriers of defense. The incorporation of two *Bt* genes, *cry*1*Ac* and *cry*2*Ab*2 in cotton variety Bollgard II is an example of complex defense barrier through stacking of two resistance genes with different modes of action making it difficult for the pests to develop resistance to two proteins simultaneously. Eventually though, invasion of the barrier by the pests should be expected, and thus as with conventional breeding, transgenic breeding will also have to remain a continuous process.

The public and scientific debate on the long-term consequences of unnecessary DNA-sequences introduced into the plant during the transformation process continues. It is feared that widespread use of antibiotic-resistant genes for markers could lead to the evolution of bacterial varieties resistant to antibiotics. Hohn *et al.* [173] has reviewed strategies employed to eliminate selectable marker recombinant DNA-sequences from the transgenic plants. Zuo *et al.* [174] effectively removed marker genes from transgenic plants using the Cre/*loxP* site-specific recombinase. Some transformation systems, now, can be performed with any selectable marker systems [175].

Consumers rightfully should have a choice to consume or not to consume food grown out of transgenic crops. The separation of a crop produced by both transgenic and non-transgenic seeds on the same farm can be costly and bear chance of mix-up and resulting costly food recall similar to Starlink corn episode. Keenan and Stemmer [176] suggest transgene deletion from the food portion (grain, tuber) of the transgenic plants with appropri-

ate recombinase to eliminate the need for identity preservation of food produced from non-transgenic varieties. The recombinase protein itself should also be rapidly degradable. Regulatory guidelines as to the acceptable level of transgenic DNA "residue" in food should assure the public safeguard.

There is also concern that pollen from a transgenic variety will drift and cross-fertilize the nontransgenic variety of the same crop in the vicinity or cross between herbicide resistant transgenics and related wild species will create "superweed". Ways to prevent transgene outflow could consist of measures to ensure its inheritance only through maternally derived genome by engineered male sterility or introduction of transgene into the chloroplast rather than nuclear genome [168] [169] [177] [178], but these techniques have potential pitfalls. Male sterility will cause problems for farmers intending to save seeds for the next crop. The transfer of 0.1% - 0.5% of chloroplast traits via pollen [179] in tobacco as well as parental inheritance of chloroplast DNA in other higher plants has been observed [180] [181]. In addition, Huang *et al.* [182] observed that transgenes can migrate from the chloroplast to the nuclear genome. Singh *et al.* [183] described a strategy based on epigenetic inheritance (imprinting) and post-transcriptional gene silencing (PTGS)/RNA interference (RNAi) that would allow all seeds from self-pollinated transgenic plants to be harvested and re-sown, without the need for specific treatments, while retaining all of the transgenes present in the parent while preventing outcrossing via either male or female gametophyte.

6. Future Scope

The overall prospects of acceptance of transgenic crops and products will depend on positive public perception of this technology, especially as the agrobiotech industry complies with regulations, conducts rigorous research on biosafety and successfully completes field trials of these crops. Like any other newly developed technology, transgenic crop agrobiotechnology industry faces its own unique challenges and hurdles, especially relating to consumer concerns on health risks and environmental safety and barriers to world-wide trade. Long term effects of GE foods must be rigorously studied but guidelines and regulations for field-testing and marketing of GM products likewise must be clearly defined to remove ambiguity and potential law suits.

There are numerous technological challenges that must be overcome while attempting to introduce specific traits into a crop. Furthermore, the high cost (approximately US$100 - 136 million [184] [185]) of developing and obtaining authorization for the commercialization of a transgenic event limits the development of transgenic crops only for selecting traits of wide interest. Agro-based companies like Monsanto, Dupont, Syngenta, Dow Agro, and Bayer Crop Science have daunting task of generating profit for share holders while they are also sensitive to the farming community and have a humanistic, consumer-oriented approach.

On positive note, numerous successes have been documented especially with regard to agronomic traits like herbicide resistance, pest resistance and drought tolerance. The high adoption rate of genetically engineered crops and controlled field trials and research in many developing countries and some EU countries are optimistic developments. The need to feed a growing world population, enhancing ability of crops to withstand climate change, and public preference for plant based industrial and pharmaceutical products should drive further research in GM plants and their production worldwide. Successful commercialization and marketing of transgenic crops and products would require mutual understanding and implementation of international standards and trade policies among nations.

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