Review Article

Recent advances in genetic manipulation of crops: A promising approach to address the global food and industrial applications

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Abstract

Continuous increase in world's population demands high food production, which has become a major challenge to the humanity. When there is sufficient amount of nutritious food to all the people there will be no problem of food scarcity. So, to increase the food production, many countries are adopting strategies of genetic engineering to enhance the crop yield. Recombinant DNA technology can be a viable source to develop genetically modified crops with enhanced resistance and improved yields to fight against malnutrition and food scarcity. With this technology, selected traits can be inserted into the plant genome, unlike traditional plant breeding, where many characters of two different crops will be combined which may lead to genetic modification at an extensive level. Present review focuses on the methods of plant transformation and outlines the scope of genetic transformation for improved crop production by transferring selected genes for biotic and abiotic stress tolerance. In addition, current study also provides information about various genetically modified crops produced worldwide and their commercialization towards various biotechnological products like GM livestock, GM microorganisms, vaccines and industrial products like bio-plastic produced from the transgenic plants.

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Introduction

Since many years, plants with desirable characteristics were being produced by employing conventional breeding methods. In this process, desirable traits will be selected, combined and propagated by continuous crossing for various generations and is a very long method, which takes up to 15 years to produce new varieties with desired characters (1). Based on the present conditions, traditional methods solely will not be sufficient to supply required food, fuel and fiber to overcome the future demands. In this approach, genetic engineering plays an important role in

increasing the productivity similar to the period of green revolution during 1960s to 1980s, which brought a great change in rural incomes and this idea is about three decades old (2). The advantage of these techniques related to the traditional breeding methods is, they not only efficiently expedite in a highly focused manner by inserting particular genes, but also prevailed the limit of sexual variance between different plant species and immensely raise the available gene pool (3). By adopting the strategy of genetic engineering, rural poverty can be decreased by increasing the food production and it encircles all aspects of agricultural production that includes high crop yield, less fertilizer and pesticide applications, increase in quality, simple processing and improved storage, better quality of the products and modern technologies to examine the condition of plants.

This field further encircles a broad range of technologies and can be used for a wide range of purposes, like generation of new plant varieties and animal communities to increase their yields, development of disease and insect resistant varieties, abiotic stress tolerant varieties, diagnosis of plant or animal diseases, increasing the livestock feed and production of plant based vaccines (4-15). Commercial transgenic crops with desired traits can be developed by the insertion of one or more new genes along with regulatory sequences or by down regulating the internal genes (16). In plant biotechnology, to control the gene expression various methods like RNA sequencing could be used (17) and in this orientation various synthetic promoters, repressors and enhancers were developed by the scientists for innate and transgenes expression regulation.

With the study of DNA structure and its replication in 1950s, research on the alteration of the genetic material was increased and the first recombinant DNA molecule was developed by Paul Berg in 1972 by combining DNA from the monkey virus SV40 with the lambda virus (18). In conventional breeding methods, a single cross between two plants generate a set of around 15,000 to 25,000 genes together, in which there will be an immense genetic change. But, by the means of modern genetic engineering methods, only some particular genes will be transformed without altering the remaining genome and this benefit the breeders to access to an ample set of new genes, which can be transformed to local, high yielding varieties (19).

Through recombinant DNA technology, the genetic material of living organisms such as animals, plants or microorganisms will be altered and the resulting organism is said to be 'Genetically modified' (GM), 'Genetically engineered' or 'Transgenic' (20, 21). In this process, the gene will be integrated in the host genome and the protein encoded by the gene will express a specific character to that plant (22). With the advances in recombinant DNA technology, many transgenic crops expressing new characters were developed and commercially released to the market. These include pest resistant maize, cotton, canola (mainly for *Bt* or *Bacillus thuringiensis*), viral disease resistant papaya, potato and squash, herbicide glyphosate resistant cotton and soybean, etc (23, 24). The first genetically altered plant was tobacco that is produced in 1983 and approved in USA and France as herbicide tolerant and an economic crop resistant to the bromoxynil herbicide (25) (Table 1). In addition, the first commercially cultivated genetically modified whole food crop was tomato (called as FlavrSavr), which has long shelf life, developed by a Californian company, Calgene (25). Besides them, various transgenic crops were in pipeline and were not yet commercially released, which have the characteristics of phytoremediation, biofortification and production of plant based pharmaceuticals like rice with immense level of carotenoid for the production of vitamin A (*e.g.* Golden rice) and bananas containing vaccines (26).

Table 1. Timeline of events featuring the present era of GM crops

Year	Achievements
1946	Scientists discovered that DNA can be transferable between species
1954	Discovery of DNA, conception of central dogma by Watson and Crick
1973	First genetic recombination experiment was conducted by Boyer and Cohen
1983	First GM plants (*Tobacco*, *Petunia*) were successfully produced
1990	China becomes the first country to commercialize GM crops
1994	First FDA approved GM crop for human consumption (Flavr Savr Tomato: Calgene, USA)
1996	First genetically modified soybean was introduced in the United States market
1996	*Bt* cotton was first approved for commercial use in the United States
2002	GM cotton was first approved in India
2002	Transgenic mustard DMH - 11 was developed
2009-2010	Bt brinjal was first commercialized on 14th October 2009, but Indian Government officially announced on 9th February 2010 that it needs further testing
2018	Genetic Engineering Appraisal Committee approved DMH - 11 for field studies

Globally, genetic improvement of the major crops has been prominently facilitated by adopting several techniques like direct gene transfer or *Agrobacterium tumefaciens* mediated gene transfer depending upon the plant species (27-33). Even though, the recombinant DNA technology was emerged in 1983, the first genetically engineered crop was commercially released in mid 1990s and till date various transgenic crops have been developed having resistance to different biotic and abiotic stresses. Even though transgenic technology has

Table 2. Various crops produced through tools of recent genetic engineering technology other than transgenesis

Plant	Technology approached	Gene	Kind of DNA modification	Achieved trait	References
Apple	Intragenesis	HcrVf2	Expression	Scab resistance	Joshi *et al.* 269
Alfalfa		Comt	Silencing Reduced levels of	lignin	Weeks *et al.* 270
Potato		StAs1, StAS2	Silencing	Limit acrylamide in French Fries	Rommens *et al.* 271
Barley	Cisgenesis	HvPAPhy_a	Over expression	Improved grain phytase activity	Holme *et al.* 272
Potato		R-genes	Expression	Late blight resistance	Haverkort *et al.* 273
Durum wheat		1Dy10	Expression	Improvement in baking quality	Gadaleta *et al.* 274
Arabidopsis thaliana	Zinc-finger nucleases	RPP4 gene cluster	Large deletion		Qi *et al.* 275
Glycine max		DCL1a/b, DCL4a/b, RDR6a, HEN1a, transgene	Knockout		Curtin *et al.* 276
Arabidopsis thaliana		ADH1, TT4	Knockout		Zhang *et al.* 277
Maize		ZmIPK1	Homologous recombination	Herbicide tolerant and phytate reduced maize	Shukla *et al.* 278
Rice		OsQQR	Homologous recombination	Trait stacking	Cantos *et al.* 279
Hordeum vulgare	TALEN	PAPhy_a	Knockout		Wendt *et al.* 280
Glycine max		FAD2-1A/B	Knockout	Improved oil quality	Haun *et al.* 281
Sugar cane		COMT	Non-homologous end joining	Enhanced cell wall composition	Jung *et al.* 282
Triticum aestivum		MLO	Knockout	Resistance to powdery mildew	Wang *et al.* 283
Zea mays	Meganuclease	Intergenic sequence	Knockout		Gao *et al.* 284
Zea mays		MS26	Knockout	Male sterility	Djukanovic *et al.* 285
Tobacco; Arabidopsis thaliana; sorghum; Oryza sativa	CRISPR/Cas	OsSWEET14, transgene	Knockout		Jiang *et al.* 286
Arabidopsis thaliana; Nicotiana benthamiana		AtPDS3, AtRACK1c, NbPDS3	Knockout		Li *et al.* 287
Arabidopsis thaliana		TT4, GAI, BRI1, JAZ1, CHLI, AP1, transgene	Knockout		Feng *et al.* 288
Oryza sativa; Triticum aestivum		OsPDS, OsBADH2, Os02g23823, OsMPK2, TaMLO	Knockout		Shan *et al.* 289
Sweet orange		PDS	Knockout		Jia and Wang 290
Rice	CRISPR/Cas9	SBEIIb	Non-homologous end joining	High amylose content	Sun *et al.* 291
Wheat		EDR1	Non-homologous end joining	Powdery mildew resistance	Zhang *et al.* 292
Rice		SBEIIb	Non-homologous end joining	High amylose content	Sun *et al.* 291
Nicotiana benthamiana	TALE activator (native TALE activation domain, VP16, GAL4)	Transgene	Control of gene expression		Geibler *et al.* 293
Arabidopsis thaliana	TALE repressor (SRDX)	RD29A, transgene	Control of gene expression		Mahfouz *et al.* 294

achieved substantial economic value relative to plant breeding, certain constraints are limiting its application in various commercially important crops that are recalcitrant to *in vitro* regeneration and genetic transformation which is to be addressed. In addition, the transgenic crops already developed were encountering disapproval and are not readily being accepted by the consumers due to food safety and environmental problems (34). The main worry of public in using transgenic crops is the incorporation of foreign DNA into the genome of the plants other than the plant's natural gene repository

to attain certain characters. So, to meet this concern, two new transformation methods were developed as a substitute to regular transgenesis called as intragenesis and cisgenesis. In both of these techniques the plants should be transformed only with the genetic material isolated from the closely associated species capable of sexual hybridization. Traditional breeding methods have been conveniently developed for gene stacking, however only a few number of single loci could be basically stacked and is a totally long process, which is paving a way for scientists to search for new methods (35). In this regard, a set of new technologies jointly called as gene editing techniques are emerging as a prominent tools in current plant biotechnology as they promote fast editing of different genes by cisgenic, mutational or transgenic methods and setting genetic engineering methods easy and simple (36) (Table 2). The following are the newly developed gene editing technologies:

1. Engineered Meganucleases (EMNs): They are double stranded DNases also called as homing nucleases that aim broad recognition sites, which stimulates effective gene targeting by double stranded break induced homologous recombination, leads to reform breaks in the DNA double strands. The engineered meganucleases adopted from microorganisms are altered to create double stranded breaks, which acts as a standard platform to direct the enzymes and exactly allow them to cleave the DNA at target site in the process of recombination (37). They are utilized to produce herbicide resistance and insect tolerance in cotton (38) and male sterility in maize (39).

2. Zinc Finger Nucleases (ZFNs): They belong to group of artificial meganucleases DNA binding proteins that promotes specific genome editing by producing double strand breaks in the DNA at particular locations succeeded by genetic modification at the time of successive repair (40). They act as distinct genomic scissors and aids in fast integration or disruption into any loci and the mutations resulted are genetic and long-lasting. In various plants like maize they are utilized to generate herbicide resistance (41).

3. Oligonucleotide Mediated Mutagenesis (OMM): They are used to induce site specific mutations with the help of chemically integrated oligonucleotides similar to the target site (42). In rice (43) and maize (44), herbicide resistance was developed by oligonucleotide mediated mutagenesis.

4. Transcription Activator-Like Effector Nucleases (TALENs): These are programmed nucleases consisting of DNA binding region of *Xanthomonas* derived effectors. They can bind to any specific DNA sequence, so when it is linked with a nuclease, DNA could be incised at particular sites. TALENs have the capability to develop important characters in an individual by the mode of target alteration of a gene family and aids in crop improvement and genome engineering (45). They are utilized to develop bacterial blight disease tolerance in rice (46), to enhance the soybean oil content (47) and to induce mutations in barley (48).

5. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9): It is an alignment of programmable nucleases comprised of an individual single-guide RNA (sgRNA) and endonucleases derived from bacteria (Cas9). It is a method to develop breaks in double stranded DNA at particular genome sites, to alter, restore or combine new genes at those particular sites (49). After cleaving the DNA with site directed nucleases (SDNs) the cell could be driven to mutate solely with limited point alterations to the gene (SDN-1) or could add a template to create more deletions or insertions in the gene sequence (SDN-2) or add a template for inclusion of distinct gene as a whole, even from a far away species (SDN-3) (49). With the development of CRISPER/Cas9, the gene knockout process has become easy and it would seem to work in nearly all microbes. Bacterial blight disease resistance in rice (50) and drought resistance in maize (51) had been developed with CRISPER technology.

Over the past few years development of recombinant technology has been increased rapidly, where in 2017, the global area of genetically modified (GM) crops was increased by 3 % i.e. 189.8 million hectares compared to 185.1 hectares in 2016, where 17 million farmers in 24 countries planted GM crops (52) (Fig. 1).
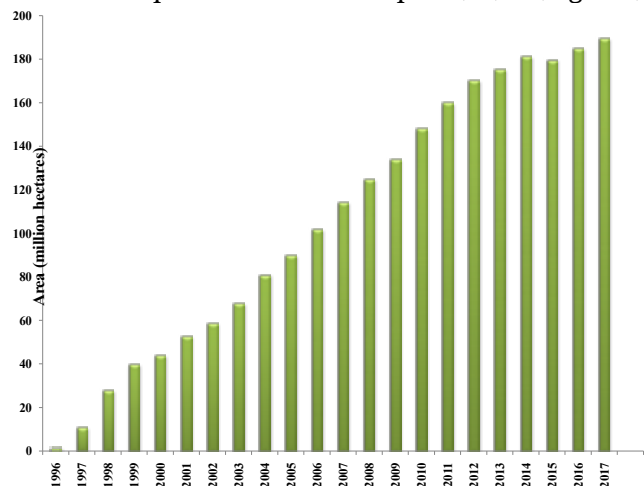

Fig. 1. Global area of genetically modified crops cultivated from 1996 to 2017

The ISAAA report mentioned that it is primarily due to greater profitability arriving from higher commodity prices, increased global and domestic market demand and available seed technologies. In the year 2017, 67 countries cultivated genetically modified crops, which include 24 countries in total that grew transgenic crops, including 19 developing and 5 industrial countries and in additional 43 non-planting countries that regulates the importation and use of biotech crops

for food, feed and processing. Out of total GM crops cultivated, about 50 % of the global area accounts for GM soybean varieties. The percentage of GM crops cultivated in the year 2017 in terms of global area were, 80 % cotton, 77 % soybean, 30 % canola and 32 % maize (52). The major producers and exporters of GM crops and their products are the United States of America, Argentina and Canada (53), where as in developing countries the largest producers are Argentina, Brazil, China and India (54). Among these countries, GM crops are prevalent in United States of America.

Among the developing countries the choice of GM crops varies, in which insect resistant cotton is the foremost commercially produced transgenic crop in Asian and African countries, whereas herbicide-resistant soybean followed by insect-resistant corn is predominant in the Latin American continent. According to the ISAAA report (52), the current production of next generation biotech crops, like anthocyanin enhanced super sweet pineapple, apples and potatoes (which will not be damaged or spoiled quickly), insect resistant sugarcane, new soybean variety with altered oil content, high amylase and increased ear biomass maize varieties contributes to food producers and consumers. In the year 2017, India occupied fifth largest position in cultivating GM crops accounting to an area of 11.4 mha after US, Brazil, Argentina and Canada (52) (Fig. 2).
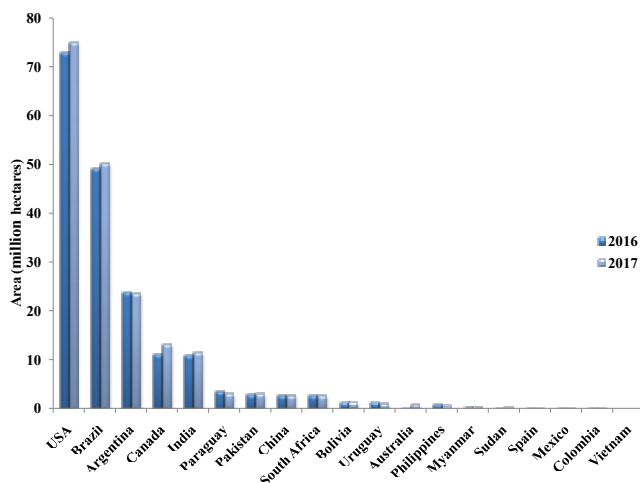

Fig. 2. Distribution of genetically modified crops among various countries in 2016 & 2017

Based on the studies on GM crops related to global socio-economic and environmental impacts 1996-2016, conveyed by PG Economics (a UK-based consultancy firm specializing in GM crops), stated that in the last two decades, GM crops contributed to $186.1 billion economic gains to about 17 million farmers, in which many of them are females and small holder farmers (55). According to the global report on food crises, in 2018 globally 113 million people in 53 countries are experiencing high levels of food insecurity in most severe food crisis (56). So, practice of modern agriculture through GM strategy can mitigate the problem of food crisis and reduce poverty in various countries.

Genetic modification and its importance

GMO (Genetically modified organisms) is described as "organisms (animals, plants or microorganisms) in which the genetic material is edited in a way that it will not be developed naturally by natural recombination or by mating (57). In this method, genes will be transferred within the same species or across the species or kingdoms and the endogenous genes can be altered, enhanced or knocked out. Food scarcity is the major problem around the world, due to the problems like increase in population, less crop yield due to biotic and abiotic stresses and unpredictable weather, which is severely affecting the farmers worldwide. The Food and Agriculture Organization of the UN (United Nations) has estimated that we need to grow 70 % more food by 2050 globally to accommodate the demand of the 9 billion estimated population (58). In this perspective, generic modification or recombinant DNA technology is a potential tool to produce biotic and abiotic stress resistant and high yielding varieties.

Methods of gene transfer

For sustainable and commercial development of transgenic plants, effective genetic transformation methods must be developed for crop improvement. To get more productive transgenic plants, the major requirements are: a potent DNA delivery system, advantageous target tissues appropriate for effective regeneration, a highly reproducible and direct regeneration system to avoid somaclonal variations (59). Alternative to *Agrobacterium* mediated gene transfer, other direct transformation methods have been developed (60, 61) like microinjection (62), protoplast and intact cell electroporation (63-66), polyethyleneglycol-mediated (PEG) transfer (67) and gene gun technology (68). Even though, many methods are available *Agrobacterium tumefaciens*-mediated gene transfer, direct DNA transfer into protoplasts by the means of osmotic or electric shock and high velocity bombardment of DNA coated microprojectiles called as the biolistic procedure are being used adequately. In these techniques, individual plant cells are targeted and are regenerated into whole GM plants using standardized tissue culture protocols.

Agrobacterium tumefaciens-mediated genetic transformation

Agrobacterium tumefaciens is a soil phytopathogen that naturally infects the plants and causes crown gall disease by transferring the T-DNA from bacterial cells into host cells through a bacterial type IV secretion system (T4SS). With the successful transformation efficiency in the host cells, *A. tumefaciens* has becomes the most suitable transformation tool to date. By the *Agrobacterium* mediated genetic transformation, any particular

gene of interest can be used to change the oncogenes in the T-DNA region of various types of binary vectors. It has the distinct capability to transfer a particular DNA segment (T-DNA) of the tumor inducing (Ti) plasmid into the nucleus of infected cells where it is then stably integrated into the host genome and transcribed, causing the crown gall disease (69, 70). It plays an important role in the fields of plant genetic engineering and molecular biology. Until now 80 % of transgenic plants were produced using *Agrobacterium* mediated plant transformation (71). At the beginning it was believed that only dicots and some monocots species can be transformed by this bacterium, but the present results has changed this aspect by demonstrating that many recalcitrant species, which are not included in its natural host range can be transformed (72, 73). Even though other methods are available, *Agrobacterium* mediated transformation method is mostly preferred over others due to its salient advantages like: reducing the transgene copy number, substantially causing minor problems with instability and transgene co-suppression (74, 75). Further it is a single-cell transformation system that does not produce mosaic plants, which are more frequent in direct transformation systems (76, 77).

Protoplast transformation

It involves the direct DNA transfer to the plant cells by using polyethylene glycol or electroporation. Once the DNA is transferred, it can be expressed or integrated stably into the genome (78). This method has the highest efficiency, where it has the possibility to attain more than 70 % transformation rate. It has been successfully used to transfer genome-editing reagents in multiple crop plants like rice, wheat, flax, potato and sweet potato (79). Some important advantages of protoplast transformation are: (i) no need of binary vector, (ii) high percentage of transformation, (iii) can be employed in maximum number of plant species, (iv) transfer of multiple plasmids with high levels of co-transformation. Apart from these advantages, some disadvantages are also there like; limited plant species are susceptible to regenerate from protoplasts, labor intensive and time consuming (79).

Particle bombardment (biolistics)

Particle bombardment is another method of gene transfer, which is also termed as particle gun, particle bombardment, particle acceleration and micro projectile bombardment. Particle bombardment is primarily described as a method to produce transgenic plants mainly in recalcitrant cereals. It is a method of transferring foreign DNA into a living cell by the means of a glass micropipette (80). This includes over-expression of certain genes and is widely used for gene transfection in mammals. Advantages of this technique are: (i) target gene can be transferred directly into a single cell, (ii) marker gene is not required, (iii) the transformed cells can be easily identified by injecting the dye along with the DNA. It uses high velocity micro projectiles to transfer substances into cells and tissues and is the only transformation technique that can be applied to almost any cell or tissue type. This method is commonly applied for genetic transformation of many organisms and plants and is employed for the plants having low regeneration capacity which show low transformation efficiency with *Agrobacterium* gene transfer in the crops like corn, rice, wheat, chickpea, pigeon pea and sorghum. Advantages of this technique are: (i) it is a simple and convenient method, (ii) genome manipulation of sub-cellular organelles, (iii) accurate transfer of DNA or RNA. Apart from advantages, some disadvantages are also there, like: transformation efficiency may be lower than *Agrobacterium* mediated transformation, costly equipment is needed and chance of gene silencing due to multiple copy insertions (81, 82). But, it has been determined that in more events, these multiple copies are aligned as a single locus and segregate in a Mendilian pattern. Over the past two decades, micro-projectile bombardment has emerged as a stable and regular technique for the transgenic plants production by leaving *Agrobacterium* host-specificity and difficulties of *in vitro* regeneration by tissue culture in many crops. With the help of biolistic approach, the complex pattern of transgene integration reported by the molecular studies can be avoided (60). This method has been used to obtain genetically engineered bean and Asparagus plants.

Various other methods of transformation

There is a need to develop more efficient and cost effective methods of plant transformation. Few of these methods are *in planta,* pollen and chloroplast mediated transformation. *In planta* transformation is a direct method without undergoing *in vitro* tissue culture work (83) and produces more number of plants with in a less time and with minimum chemical requirements (84). The two important methods of *in planta* transformation are vacuum infiltration and floral dip and both were promisingly been applied in vegetables, oil seeds, cereals and various other crops (85). Coming to the chloroplast transformation method, it has several advantages like multiple gene transfer in one transformation event, enhanced level of gene expression and with no gene silencing and pleiotropic effects. With the help of tobacco chloroplast genome, more than fourty genes have been firmly integrated and expressed to confer essential characters or expressed maximum levels of biopharmaceuticals and vaccine antigens (86). This method of transformation has been successful in several main crops like soybean, cotton (87, 88) and carrot (87). In addition to the above two methods, pollen transformation is also a potent method to transfer

various foreign genes. Pollen transformation, succeeded by stigma pollination with transformed pollen grains and consequent choosing of genetically transformed plants and seeds is a fast and simple alternative method to produce transgenic plants by avoiding *in vitro* culture (88). Similar to other transformation methods, *Agrobacterium tumefaciens* could be applied as vector or the DNA could be directly transferred to the specific region and is capable of producing genetically transformed plants in less time. In case of *Petunia hybrida,* transgenic plants are produced by pollinating pollen grains next to vacuum infiltration with *Agrobacterium tumefaciens* (89). To produce transgenic maize plants, ultrasonication was applied to transfer plasmid DNA into maize pollen grains, which were later utilized for pollination of flowers (90). In case of *Brassica juncea*, sonication facilitated addition of *aroAM1* gene adopting glyphosate as a selectable promoter was applied successfully (91).

Achievements of transgenic technology

Modern biotechnological techniques have rapidly expanded the horizons of plant breeding and crop improvement. In this process, development of different plant transformation techniques to produce various biotic and abiotic resistant crops to address several problems in agriculture led to the study of structure and function of desired genes. In the initial stages, single gene of interest was transferred into plants, but now-a-days with the help of developed advanced procedures, multiple genes against one metabolic pathway were successfully integrated (92). The major ways adopted to produce improved transgenic plants are communicated below.

Development of biotic and abiotic stress resistant crops

A major loss in the crop yield is due to various biotic and abiotic factors that affect the plant growth which limit their geographical distribution. To address these problems, genetic transformation is an important mechanism to impart disease resistance and enhance crop yield that leads to a new revolution in crop improvement. The below sections will discuss about some of the transgenic crops developed against biotic and abiotic stresses.

Herbicide tolerance (HT)

Weed control is a regular problem in agricultural fields, where they not only compete with crops for nutrients, water, sunlight and area but also block irrigation and drainage systems; dispersal of weed seeds into crop harvests and decrease the crop quality and yield (93). Herbicides are used as a primary means to control weeds in current agriculture, even though their wrong application directed the development of herbicide tolerant weeds (94). Conventional agricultural systems can only use 'selective' herbicides that do not harm the crop but are not effective in removing all types of weeds but during this period some of the weeds become tolerant to few normally utilized herbicides (95). Several crops have been genetically modified which are resistant to non-selective herbicides. Agrobacterium-mediated transformation and particle bombardment methods are the most widely used methods in the production of herbicide-resistant crops (96). In addition, other biotechnological methods like mutagenesis and *in vitro* cell culture can also be used to develop herbicide tolerance (97).

The transgenic crops developed consist of genes that enable them to degrade the active ingredient in a herbicide and rendering it harmless. Farmers can there by easily control weeds during the entire growing season and have more flexibility in choosing spraying times and these herbicide resistant crops also facilitate low or no tillage cultural practices, which are considered to be more sustainable (98). Another advantage is that farmers can manage weeds without switching to some of the environmental susceptible herbicides. The global herbicides utilization in HT crops has increased during the time period 1998 to 2013, where increase in rate (kg/ha) of active ingredient in HT soybean was 64 % relative to 19 % in traditional soybean variety (99).

Herbicide resistance has been developed in many crop species, such as oilseed rape, maize, soybeans, sugar beet, fodder beet, cotton, rice and coffee (100-105). The first herbicide-tolerant GM plants commercially grown were glyphosate-tolerant soybeans (106, 107). The gene that imparts herbicide tolerance is derived from the soil bacterium *Agrobacterium tumefaciens* that encodes an EPSPS (5-enolpyruvylshikimate 3-phosphate synthase) is not affected by glyphosate. Presently, 36 different weed varieties tolerant to EPSPS inhibitor (major transgenic herbicide), glyphosate and about 159 varieties tolerant to ALS-suppressing herbicides have been developed (108). Today, at least 36 weed species have evolved resistance to glyphosate, EPSPS inhibitor (the main herbicide in transgenic HR crops), and at least 159 to ALS-inhibiting herbicides (the main group of herbicides in non-transgenic HR crops) (109).

In the USA and Canada, Glufosinate and bromoxynil tolerant varieties of oilseed rape have been successfully developed (110). In the year 2002, herbicide tolerant GM crops like 15% of maize, 59% of upland cotton and 81 % of soybean (111, 112) were cultivated in the USA. In addition, 66 % of canadian oilseed rape and 95 % of Argentine soybean herbicide tolerant GM plants were also produced during the same period (113). From 1996 to 2017, most of the planting area of biotech crops was occupied by herbicide tolerant crops. In the single year 2017, herbicide tolerant crops inhabited 88.7 million hectares or 47 % of the 189.8 million hectares of the total biotech crops planted globally (52). Based on the studies

carried out by the council for agricultural science and technology, it was assessed that the environment gains by the adoption of HT crops. For example, in the US, no-till soybean acreage has increased by 35 % from the period of introduction of herbicide tolerant soybean. The same situation was observed in Argentina, where 98 % of soybean fields were planted with herbicide tolerance varieties. Since the early 21 years of commercialization (1996-2016), profits from HT crops were estimated at US$ 89.02 billion, global biotech crop value of US$ 186.1 billion with 47.8 % and in the single year 2016 at US$ 8.44 billion or 46.4 % of global value of US$ 18.2 billion (52).

Insect pest resistance

Development of genetically engineered pest resistant crops reduced the usage of broad-spectrum insecticides, which provide a safer, more biologically sustainable way of managing insect pests. Various pests attack plants and cause immense loss and low product quality that leads to major global food security and every year 25 % of food crops were destroyed globally (114). Experience has shown that crop yields can be enhanced or increased with decreased spraying of pesticides. For example, European corn borer (ECB) (*Ostrinia nubilalis*), annually generates a loss up to 2 billion dollars in the USA alone (115). *Bacillus thuringiensis* is a soil-borne gram positive bacterium discovered in diseased silkworms by Ishiwatari in 1901 to control various pests (116). It produces a protein called as *Bt* toxin, which is produced in an inactive, crystalline protein form that is toxic to various herbivorous insects and have high insecticidal activity at very low concentrations. Whenever insects consume this, the protein is converted to its active toxic form (delta endotoxin) and destroys the gut of the insect and kills it (117), because this particular protein is active form in alkaline condition inside the gut of the insect, where as in humans this protein will be digested inside the stomach due to its acidic nature. *Bt* preparations are commonly used in organic agriculture to control insects, as they occur naturally and safe for humans. More than 100 different variations of *Bt* toxin have been identified in diverse strains of *Bacillus thuringiensis* and they have different target insect specificity. For example, toxins classified under *Cry1a* group target *Lepidoptera* (butterflies), and toxins in the *Cry3* group are effective against beetles (118-121).

In China, cultivation of cotton bollworm resistant cotton varieties decreased the application of chemical pesticides that not only reduces the adverse effects on the environment but also minimizes the negative effect of pesticides on farmer's health (122, 123). Due to the reasons of improved health benefits and high yield by low pesticide use, commercialization of *Bt* cotton has been increased globally, mainly in Asian countries like India (124) and China (125). In India,

transgenic rice varieties resistant to *Scirpophagain certulas* walker (yellow stem borer) were developed (126) which eradicated yield loss caused by *Lepidopteran* insects, that accounts to Asia's 2 to 10 % annual rice yield of 523 million tons (127). Among the different GM insect resistant varieties developed, transgenic rice variety exhibited high resistance to yellow stem borer (128). In 2017 (22nd year of GM crops commercialization), 24 countries cultivated 189.8 million hectares of GM crops compared to 185.1 million hectares in 2016 with a raise of 3 % or 4.7 million hectares (129).

Till date, more than 700 *Cry* gene sequences associated with crystal proteins have been identified (130) and these proteins were reported to eradicate *Coleoptera*, *Lepidoptera*, *Hymenoptera* and *Diptera* pests in the fields (131). In addition to these, alternative anti-insect genes like plant agglutinins and plant protease inhibitors, non-*Bt* genes from bacteria (*Pseudomonas entomophila*, *Serratia entomophila* and *Morganella morganii*) and other genes from fungi [*Beauveria bassiana*, *Metarhizium anisopliae* (countering locusts or beetles) and *B. brongniartii*] were also identified (132). But, most of the insect pests are not affected and regulated properly by these genes. Hence, more research is needed to analyze most potent insect resistant genes.

Disease resistance

Continuous efforts are being made for the development of alternative approaches in plant disease management to reduce the application of chemical fertilizers. Bacterial, viral and fungal diseases are absolutely adjustable for natural adaption and effect the plant growth. Amongst the various approaches, resistance breeding has produced authorized information and had been used extremely. In normal environment, complex systems of defenses in plants operate at various zones to get protection from many diseases (133). Now-a-days, interpretation of these defensive pathways has emerged as a specific area of research in the field of plant molecular biology and will be a developing concept to study the multiple interactions among initial defenses and distinct disease resistance (134). Plants developed through breeding techniques ('R' genes or Resistance genes), may not exert disease resistance to some pathogens due to their developed pest resistance and may finally result in on set of diseases (135). The traditional conventional breeding methods alone are not enough to control the pathogens because of the in adequacy of suitable crop variations and in spite of such breeding techniques identification of resistant genes has been one of the main targets from many years (136, 137). So, the present conditions demand the identification of variations towards the biotic stress by the recognition of genes across the species.

From over the past two decades,

significant work has been made in plant disease management by the means of genetic engineering. Besides this, many molecular approaches have been evolved to resolve various plant-pathogen systems and correlated disease-resistance genes. With the advanced plant transformation techniques, promising genes can be transferred to produce disease resistant plants and outcome has been achieved by producing various disease resistant transgenic crops. One such gene is the *Bs2* gene (Bacterial spot resistance gene) from pepper, which has been successfully used to accomplish resistance against agriculturally serious bacterial spot disease in tomatoes (138).

Two main bacterial blight resistant genes, *Xa21* (*Xanthomonas Oryzae PV. Oryzae Resistance* 21) and *Xa13* (*Xanthomonas Oryzae PV. Oryzae Resistance* 13) were incorporated into the famous basmati rice variety Pusa Basmati 1 and in the year 2007 it was released into the market as 'Improved Pusa Basmati 1' variety (139). Sundaram et al. (140) incorporated three bacterial blight resistant genes *Xa21, Xa13* and *Xa5* (*Xanthomonas Oryzae PV. Oryzae Resistance* 5) in the best Samba Mahsuri rice variety and named it as 'Improved Samba Mahsuri', which is a high yielding and bacterial blight resistant variety (141). Few examples of other disease resistance genes are oxalate degrading enzyme, *OXDC* (*oxalate decarboxylase*), expressed in tomato, lathyrus, soybean and tobacco had shown high resistance to the pathogen *Sclerotinia sclerotiorum* that uses oxalic acid at the time of host settlement (142, 143). By using the RNA interference strategy (RNAi), which is a main regulatory mechanism for gene expression and anti-viral protection in eukaryotes, the defense mechanism can be improved against the pathogens in various crops (144-146). Virus infection is also one of the major problems in many crops that result in significant yield loss. In the process of management the viral coat protein has been used to bring resistance against viruses and this is one of the best methods used in genetic engineering. Some of the virus resistant plants brought to the market are PYV tolerant varieties of potato (potato Y virus) or PLRV (Potato Leaf Roll Virus) (147). Virus resistance was also attained by the use of sense and antisense RNA by governing the replication associated protein (AC1) of African Cassava Mosaic or the *C1* gene from the Gemini virus and resistance can also be shown by expressing the defective viral Movement Protein (MP) (148). One of the new ways to develop virus resistant plants is Post Transcriptional Gene Silencing (PTGS), which produces broad range of virus resistant plants. Example of PTGS method generated resistance is against Gemini viruses in plants (149). By the method of RNA silencing, transgenic virus resistant papaya was produced against Papaya ring spot POTYVIRUS (150).

Transgenes can be expressed in whole plants to promote disease resistance by the constitutive expression of AMPs (Antimicrobial peptides) under CaMV35S (Cauliflower mosaic virus 35S promoter) or ubiquitin promoters to increase crop yield. By this way, endo-1, 3-beta-d-glucanase gene from potato was expressed in tea plant to develop blister blight resistance in tea (151). Likewise, *Xoo* bacterial blight resistant transgenic rice was developed by the over-expression of *OsPR10a* gene (152). In another study, constitutive expression of *Tfgd2* (*Trigonella foenum-graecum* defensin 2) - *RsAFP2* (*Raphanus sativus* antifungal protein 2) fusion gene under CaMV35S promoter in transgenic tobacco plants displayed improved insect and disease resistance against *Phytophthora parasitica* var. *nicotianae*, *Rhizoctonia solani* pathogens and *Spodoptera litura* pest (153). Expression of *SniOLP* (*S. nigrum* osmotin-like protein*)* and *RsAFP2* (*Raphanus sativus* antifungal protein 2) genes in transgenic peanut plants under separate CaMV35S promoters exhibited improved resistance to late leaf spot disease caused by *Phaeoisariopsis personata* (154). Plants developed to attain eminent levels of salicylic acid also displayed improved disease resistance (155). In transgenic plants, different antibacterial proteins from various sources other than plants also exhibited resistance to bacterial diseases (156).

Abiotic Stress tolerance

For three decades, the molecular biology approaches have extended the chances of directly modifying the genomes of higher plants to alter their metabolism to improve the growth and yield under unfavorable environmental conditions to furnish the human requirements (157-160). Since the 19th century, the carbon dioxide concentration in the atmosphere has been constantly increasing and currently reached to 400 ppm at some observed locations (161) that is increasing the global warming which has become a major issue in the last few years. Even though it is crucial to accomplish raising carbon dioxide concentrations with extreme weather conditions like drought, heavy rain, or low and high temperatures, these are attained with increased frequency (162). Agriculture was entrenched by acclimatizing plants to grow in particular climatic conditions as the yield relies upon the climatic conditions and usually reduces at the time of extreme weather (163). Further, advancement of saline or drought conditions due to different human activities are decreasing the available cultivated land and yield, but there is demand of requirement of higher yields (164).Worldwide, more than 50 % of the crop yield was being affected due to abiotic stress (165). Previous reports states that, globally about 800 million hectares of lands are saline, which covers about 6 % of the total area and in addition to that salinity has damaged about 20 % of cultivable land (166). The practice of irrigation

made certain land available for cultivation and according to current estimation over 20 % of the global cultivable land is irrigated which contributes 40 % of food and feed (167), but 50 % of land was under salinity (168). Different surveys presumes that there is a threat of losing 30 % of cultivable land in a period of 25 years and about 50 % by 2050 (169) and according to the recent reports, presently 70 % of the global fresh water is utilized by agriculture (167). Hence, productive usage of water resources is important for future agricultural practices.

Even though, traditional plant breeding methods afford marginal support, genetic engineering techniques provide rapid and efficient strategy methods for managing stress related problems especially in improving plant stress tolerance. In this approach, one of the best classical ways of enhancing stress tolerance is to evaluate and distinguish the resistant genes and transfer them to higher plants. To avoid the stress conditions, plants have adapted various methods to overcome the stress challenges by choosing either a system which makes them to sustain from the adverse effects or developing certain growth habits. At transcriptional and translational stages, hundreds of genes and their products react to the abiotic stresses (170). Depending on the function, genes correlated with abiotic stress are categorized into three groups: Functional proteins, signaling factors and transcriptional factors. Relative to signaling factors which include proteins engaged in signal transduction regulation, functional proteins involve genes that regulates abscisic acid (ABA) synthesis, antioxidant protectants, reactive oxygen species scavenging proteins, chaperons, LEA proteins and heat shock proteins (HSPs), which are engaged in giving protection. Transcriptional factors (DREB1/CBF, AP2/ERF, DREB2, NAC, MYB/MYC, basic leucine-Zipper proteins and Zinc-finger proteins) are connected with integrity of ion and cell homeostasis. At the molecular stage, abiotic stress tolerance could be developed by gene transfer by modifying the aggregation of osmoprotectants, chaperones synthesis, superoxide radical scavenging mechanisms, compartmentation or exclusion of ions through competent symporter and transporter systems (171-175). Among different stress factors, drought and salinity are considered as the most serious problems decreasing the agricultural production on an overall range. The global environmental conditions are expected to continue leading to increased both salt and drought stresses.

Drought

For more than 20 years, various scientists have performed immense research on the structural, biochemical, physiological, molecular regulation and on morphological traits to disclose the methods of drought responses of plants. The plant reaction to the drought stress is a multiple process comprising different genes and signaling pathways. Genes engaged in these reactions could be arranged into two major classes: single function genes and regulatory genes based on their biological function (176). The single function genes encode enzymes associated with the accumulation of osmolytes, proteins and enzymes scavenging oxygen radicals (ROS), proteins associated with the uptake and transport of water and ions (ion transporters, channels), and proteins involved in lipid biosynthesis (177). Even though, many genes have been recognized that they can impart drought tolerance in plants only few drought tolerant crops have been developed, because the progress has been restricted to produce such tolerant crops for field conditions or commercialization. The first drought tolerant transgenic plant is MON 87460, a maize variety developed in the year 2009 by Monsanto company and was first sown in 2013 in the country US, which increased the yield 5.5 fold from 50, 000 ha in 2013 to 2,75,000 ha in 2014 (178). This variety expresses cold shock protein B (CSPB) from *Bacillus subtilis* to provide drought tolerance. This protein in MON 87460 variety manages normal cellular functions under drought stress conditions by retaining RNA stability and translation (179). Further, the over-expression of CSPB was noted to impart stress tolerance in *Arabidopsis* and rice (180). Drought tolerant transgenic crops like rice, maize, canola and cotton were develloped in fields in the years 2009 to mid-2011s (181). Most of the new experiments were performed in rice, which is a wonderful model variety and is one of the most important crops worldwide. Stress responsive NAC1 (*SNAC1*) an NAC-type transcription factor, is particularly inferred in guard cells under drought stress conditions. Over-expression of *SNAC1* in rice developed an enhanced drought tolerance under serious drought conditions at reproductive stage in the field, where 22-34 % higher seed setting was observed (182). Similarly, over-expression of AVP1 in tomato, rice and *Arabidopsis* improved plant efficiency under drought stress and salt conditions (183, 184).

Salinity

Another major abiotic stress is salinity stress and in India about 6.73 million ha is affected with salinity (185). This has encouraged scientists to develop various techniques to produce high yielding transgenic plants. Various studies stated that the salt tolerance is firmly associated with the capacity of maintaining ion homeostasis under salinity. It was reported that salt tolerant plants are capable of tolerating other stresses besides drought, freezing, heat and chilling (186). Using modern technology, already, transgenic plants were developed in many crops for abiotic stress tolerance which includes tomato (187), tobacco (188), rice (189), *Arabidopsis thaliana* and *Brassica napus* (190) and cotton, maize, oilseed rape and wheat (191, 192). These GM plants retained high

photosynthetic ability with maximum levels of photosynthesis-associated enzymes. Currently, a gene encoding aquaporin (NtAQP1) was recognized in tobacco (*Nicotiana tabacum*) and exhibited to give protection against salinity stress in transgenic tomato (*Solanum lycopersicum*) (182). NtAQP1 plays a key role in preventing shoot or root hydraulic failure by increasing the water use efficiency to promote salt tolerance. Based on previous literature, it was reported that glutathione (GSH) plays a key role in antioxidant defense system in plants and rise in glutathione synthesis including GSH/GSSG ratio has been observed to be associated with stress tolerance (193). The glyoxalase pathway comprising glyoxalase I (gly I) and glyoxalase II (gly II) enzymes are required for glutathione based detoxification of methyl glyoxal (MG). GM tobacco plants over-expressing glyI and glyII enzymes were developed and they were reported to have high metal and salinity tolerance than non GM crops (194, 195). In the recent findings, they observed that over-expression of rice gly II gene in rice exhibited resistance to toxic levels of methylglyoxal and NaCl compared to the non GM plants (196). Asif et al. (197) reported that transgenic plants incorporated with *AtNHX* (*Arabidopsis thaliana* Na(+)/H(+) exchanger 1) gene are highly resistant to high salt concentrations and water loss than the wild type plants and the accumulation of proline and salt was higher in the leaves of transgenic plants compared to the wild type plants. Transgenic groundnut with enhanced drought and salt tolerance was developed by over-expression of *AtNHX1* (*Arabidopsis thaliana* Na(+)/H(+) exchanger 1) gene (197) Ying-Hui Guo et al. (198) reported that GhZFP1 (*Gossypium hirsutum* zinc finger protein 1), a new CCCH-type zinc finger protein isolated from salt induced cotton, improves salt tolerance and fungal disease resistance in transgenic tobacco by combining with GZIRD21A (GhZFP1 interacting and responsive to dehydration protein 21A) and GZIPR5 (GhZFP1 interacting and pathogenesis-related protein 5). These zinc fingers are a super family involved in various factors of plant growth and development.

Waterlogging

The major threats to food security are floods and droughts (199), where the agriculture fields are usually flooded by severe or huge rainfall for a period of time. Around the world, during the period 2006 to 2016, two-thirds loss of crops and destruction are due to floods, with a loss of billions of dollars (199). Depending on the height of the produced water column, flooding could be categorized as waterlogging, when it is depthless and masks the root only, or over flow, where water fully masks the aerial parts of the plant tissues (200). The two kinds of floods disturb the oxygen movement from the air to the plant tissues (201), generating a common situation called as hypoxia (<21% O$_2$) (200).

Currently, flooding conditions like waterlogging, submergence, anoxia and hypoxia were examined widely in various plants, primarily in Arabidopsis and rice, to analyze molecular components that are capable of playing role in resistance to flooding. The incorporation of ethanolic fermentation pathway is studied to be a crucial element of reactions, which are obtained in rice and in other plants contrary to flood stress (202, 203). Two different methods are been tried to recognize the inhibiting factors in response to waterlogging. First one is the individual candidate genes under-expression, *e.g.* sense and anti-sense constitutes for ethanol synthesis and second is the transcription factors over-expression (204). It was expected that the two methods are advantageous in converting the extended term modification reaction to less oxygen stress.

Conversion of pyruvate to ethanol called as ethanolic fermentation is comparably simple method, which involves two enzymes, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH). Cloned PDC and ADH availability will increase the interest of molecular biology scientists to use them for transgenic experiments. In relation to this, the primary outcome of transgenic rice consisting of cotton ADH cDNA proposed that PDC over-expression will not exhibit high resistance to submergence. In a study (205), Taipei-309 transplanted with pdc1 associated to a constitutive 35S promoter has shown 3 fold increased activities of PDC and ethanol production when exhibited anoxia related to non transformed control plants. In contrary to this, another study (206) examined Taipei-309 transformed with pdc1 where the two transgenic lines had two fold high PDC activities and showed 43 % high percentage of ethanol production, but the durability of seed lines which were anoxia exposed was less compared to non-transformed plants. Transgenic cotton plants transformed with ADH cDNA directed by the 35S promoter exhibited ten to thirty fold enhanced ADH activity and an appreciable increase in the ethanol fermentation rate (207).

Cold stress

Low temperature has enormous effect on the geographical distribution and survival of the plants. Relying upon the period and severity of stress it influences a variety of cellular metabolisms in the plant cycle. Through various studies it was determined that the primary location of freezing damage in plants is the membrane systems (208, 209). Different subtropical and tropical region species are damaged or killed by non-frost low temperatures and show different freezing injuries like necrosis, chlorosis or growth delay. Relative to this, frost resistant varieties are capable to cultivate at cold temperatures, but numerous forms of damage to

the membrane will occur as a result of freeze generated cellular dehydration inclusive of lamellar-to-hexagonal-II phase transitions, expansion-generated-lysis and fracture jump lesions (210). Adapting living cells to freezing temperatures is an activity of altering the membrane lipid content by enhanced unsaturated fatty acids.

The use of genetic engineering in various crops by the incorporation of genes encoding cold resistant metabolites and proteins are found be a substitute to overcome the low-temperature abiotic stress. In this condition, helicases are known to play a key role in cold sensitivity in plants. Plants have more number of DEAD-box RNA helicase genes relative to other living things (142). Among the helicases, LOS4 encoded by the Arabidopsis osmotically responsive genes, is known to be vital for attaining tolerance to freezing and chilling in plants (143). Further, Arabidopsis nucleoporin AtNUP160, SAR1 (suppressor of auxin resistance1) also regulates RNA export and is important for developing freezing and chilling tolerance (147). A group of genes that encodes cold regulated (COR) proteins, were utilized by various researchers to recognize Arabidopsis transcription factors family called as generally dehydration responsive element-binding factors (DREB) (DREB1B, DREB1C and DREB1A) or C-repeat binding factors (CBF) (CBF1, CBF2 and CBF3) (211). The over-expression of CBF1/DREB1b and CBF3/DREB1a increases the cold resistance by promoting COR (cold regulated genes) genes and it further leads to various biochemical alterations like aggregation of proline and sugar (212-215). Transgenic tomato (*Solanum lycopersicum*) transformed with CBF1 cDNA within the regulation of a CaMV35S promoter exhibited enhanced resistance to salt, drought and chilling but still showed adverse effects like decreased fruit set, less number of seeds per fruit and dwarf nature (216). In a study (217), genetically engineered tomato with ring zinc finger protein (RDCPt) derived from hot pepper (*Capsicum annum*) developed enhanced resistance to cold in transgenic plants relative to non-transgenic plants. Over-expression of OsMYB3R-2 in transgenic *Arabidopsis* showed enhanced resistance to freezing while exposed to - 80^0 C for 10 h (218).

Genetically transformed tobacco plants which are over-expressed with glycerol-3-phosphate acyltransferase (GPAT) chloroplast gene from *Cucurbita maxima* (squash) and *Arabidopsis thaliana* exhibited high unsaturated fatty acids number and parallel reduction in chilling susceptibility. A cold sensitive nucleic acid binding protein, a zincfinger consisting glycine rich RNA binding protein derived from *Arabidopsis* represented as atRZ-1a is upregulated by cold stress and genetic studies helps in attaining freezing resistance (219). The *Arabidopsis* nucleoporin AtNUP160 suppressor of auxin

resistance1 (SAR1) also regulates RNA export, and is important for freezing resistance and chilling (220). Pramanik and Imai (221) stated that, TPP (trehalose-6-phosphate phosphatase) genes expressed in rice are induced by cold. The over-expression of TPP genes and TPS (trehalose-6-phosphate synthase) increased the aggregation of trehalose and resistance to cold stress in transgenic tobacco and rice (222-225).

Nutritional quality improvement

By genetic modification techniques, the nutritional quality and food content of crops can be improved and among the different crops developed cassava and rice are one of the major areas of interest for GM foods. Malnutrition is the major sustaining problem in developing countries, where most of the people depend on a sole crop like rice as major source of their diet (226). Rice is the main crop for nearly half of the people but it is not a good source of vitamin A (227). Globally, every year around 250,000 to 500,000 malnourished children are suffering from blindness due to vitamin A inadequacy, in which 50 % of them die annually (228). Ingo Potrykus and Peter Beyer in collaboration with International Rice Research Institute (IRRI) developed a new rice variety, containing β-carotene in its grains, which is a precursor to vitamin A (229). It has taken 25 years to develop and test the rice varieties having adequate amounts of β-carotene, which could eradicate the mortality and morbidity due to vitamin A deficiency (227). This "Golden rice" variety was inferred substantially to reduce blindness caused due to vitamin A deficiency (226). Vitamin A deficiency due to poor intake of diet and food scarcity results in development of major health problems estimated to cause 1.9 to 2.8 million deaths every year, and among them severely affected are women and children below 5 y (227). Further research is going on to develop new iron rich golden rice variety (226). Cassava is another crop, which is altered to enhance the nutritional content to provide healthy diet in developing countries. This starchy food is consumed by most of the people in tropical Africa, where 40 % of the calories come from it (230). Developed GM cassava variety is pest tolerant and consists of vitamin A, proteins and high mineral content, which can avoid childhood blindness, anemia, infections caused due to impaired immune systems (230). Thus, it is more reliable and staple food for the people of tropical Africa. In addition to cassava, other food crops like GM maize were developed by inserting a *cordapA* gene (*Corynebacterium glutamicum* dap A promoter) from a soil bacteria *Corynobacterium glutamicum* to produce increased lysine (LY038) content. Increased production and aggregation of free lysine in the genetically modified corn kernel led to the increase in body weight, feed conversion and body yields of experimental poultry in comparison to animals fed with lysine augmented

diets and higher than those fed with common maize diets (231). In the experiment conducted on rats, lysine enriched GM maize variety derived by the insertion of a gene from potato is also safe as common maize varieties (232). Soybean variety M703 developed for enhanced protein levels contains more digestible amino acids like methionine, lysine, threonine and valine and was experimentally proven in cockerels that it has major level of metabolizable energy than traditional soybean food (233). Narrow-leafed lupin (*Lupinus angustifolius*) which is expressing methionine-rich sunflower albumin was reported to increase methionine content twice compared to the others. If genetically modified high-methionine lupine varieties are provided to the broilers diet, supplementation of additional methionine consisting of 25 % lupin food could be decreased by 0.6 g/kg (234).

Genetic modifications of plants to develop commercial products

Biodegradable plastics

Naturally plants produce different polymers, like cellulose or starch and are been utilized for the plastic synthesis. Furthermore, new plastics such as polyhydroxyalkanoates (PHAs) were too produced from the plants. One of the promising advantages of transgenic crops is the production of biodegradable plastics (235) particularly PHB (polyhydroxybutyrate) and PHV (Polyhydroxy-Valerate). Plants may be treated as renewable, adaptable and comparably sustainable sources of plantibodies or edible vaccines (236, 237), fatty acids and new oils (238, 239) and biodegradable plastics. Harmony among the enzyme information and the genes conferring to PHA production in bacteria and plant metabolic engineering approaches would be essential for the improvement of crops that synthesize biodegradable plastics. Transgenic varieties of cotton, corn, and mustard have been genetically engineered to produce first plant based synthesized plastic compounds in the world (240). For the production of PHAs on large scale at low cost relative to artificial plastics has emerged from the presentation of PHA aggregation in transgenic *Arabidopsis* plants expressing bacterial PHA biosynthetic genes.

Developed biodegradable plastics can be degraded completely in composters or in sewage treatment plants using naturally occurring microorganisms. They don't leave any toxic, distinct or apparent waste after degradation. Such type of plastics, duly industrialized, may supply a green alternative to conventional petrochemical plastics like polyethylene, polypropylene and polystyrene and act as a source of crop based plastics. These can be synthesized from a renewable source like plants fossil material, which are biodegradable. Recently, Michigan state university scientists presented a new approach to produce economical biodegradable plastics by using an ancient microorganism under sunlight. They conducted an experiment on cyanobacteria that use sunlight to produce sugar naturally and genetically designed them to uniformly flow the sugar into the surrounding salt water medium. The treated biomass consists of nearly 30 % of bioplastic, which is four times more than the other identical experimental systems and the production rate was around 20 times faster (241). This reduces the plastic production from the fossil fuels and minimizes the negative effect of plastic on the environment (241). Researchers are focusing on genetic modification of cyanobacteria, which is also called as blue green algae to produce PHA (polyhydroxyalkanoates), which are promising raw materials for bioplastic production. Scientists from the RIKEN center for sustainable resource science also worked on blue green algae, developed a cyanobacterium strain, which yields triple amount of enhanced bioplastic polyhydroxybutyrate (PHB) than the normal strain (242). The species of cyanobacterium known as *Synechocystis* begin to generate PHB when nutrients like nitrogen become deficient. This metabolic adoption aids the survival of cyanobacteria under low resource conditions, but under the normal conditions the organisms will not generate adequate amounts of PHB for economical applications. To increase the production of PHB, scientists have engineered a *Synechocystis* strain that has enhanced expression levels of Rre37, a regulatory protein which is involved in sugar metabolism at the time of nitrogen scarcity. Genetic and metabolic analysis of Rre37 showed that, it promotes the conversion of glycogen, a sugar storage molecule into PHB (242). They further stated that, a novel regulator in Rre37 was found that activates bioplastic production in cyanobacteria. In addition to this, the same scientists earlier recognized one more protein i.e. SigE, which is involved in bioplastic production.

Biopharmaceuticals and Edible vaccines

Plants have the potential to produce peptides and biopharmaceutical proteins as they can be transformed efficiently and serve as a cheap economic source of protein. To produce recombinant pharmaceuticals in plants two different transformation strategies were commonly applied (243-247). In the first method, transgenic plants are produced by *Agrobacterium*-mediated transformation, particle bombardment or other regular transformation techniques and the second technique is to infect non transgenic plants with recombinant viruses that express transgenes at the time of replication in the host (248-250). Tobacco was the first plant to be genetically engineered and has the advantage of being used as a plant biopharmaceutical since the methods for gene transfer and expression are well established in this plant. One more reason is it can

be cultivated several times per year and produces large amount of biomass compared to other plants (251). In a pioneering study (252), for the first time transferred a chimeric gene of nopaline synthase and human growth hormone into sunflower and tobacco plants by using the Ti plasmid. Soon after, mouse monoclonal antibody was synthesized and assembled into tobacco leaf sections (247). Similar to bioreactors, plants can produce high amounts of recombinant proteins, which are not contaminated with any microorganisms of humans or animals and can be stored without chilling at low cost. Using this strategy, many recombinant proteins have been secreted in plants and the protein based pharmaceuticals production has switched from mammalian, fungal and bacterial cultures to plant cell cultures and plants (253-255). Now, different commercialized reagents and enzymes produced from plants were available. For example, type I collagen, that can self-assemble into fine homogenous fibrils, were synthesized in plants (256) similarly bovine trypsin was synthesized in maize and TrypZean (Sigma-Aldrich) has been in the market since 2002. One more example is human lysozyme and lactoferrin, which were synthesized from rice (257, 258). The process of producing plant based biopharmaceuticals from transgenic tobacco or carrot cells has been developed by Protalix, an Israeli company (259, 260). From Food and Drug Administration (FDA) of the United States, Protalix and its partner Pfizer acquired permission for taliglucerase alfa production for Gaucher's disease. Next, for the first time, transgenic plant derived biopharmaceutical, hirudin, is now being commercially produced in Canada (261).

Edible vaccines act as an alternative to traditional vaccines and can overcome the restrictions of conventional vaccines. Production of vaccines in plants was first undertaken in the year 1989 (247). The idea of using transgenic plants as platforms to produce and deliver subunit vaccines was brought by Dr. Arntzen and his colleagues (262, 263) and confirmed that this approach may overcome the constraints in conventional vaccine production (264). Plants that have been selected to use as bioreactors are potato, tobacco, rice and corn. The first subunit vaccine was produced in tobacco plants by expressing surface protein antigen of *streptococcus mutants* (264). They also began the production of hepatitis B and heat liable toxin B subunit in potato plants and potato tubers (264). Since, plant based vaccines are easy to handle, cost effective, easy production on large scale and also avoid difficulty in storage, this process may be a reasonable substitute for vaccine production (265-267). Till date, many transgenic plants have been used to synthesize four different types of vaccines *viz*. viral vaccines, bacterial vaccines, immune contraceptive vaccines and parasite vaccines (268).

Conclusion

As the global population is expected to reach 9 billion by 2050, adoption of new crop improvement technologies is crucial to face the upcoming problems in future. In this aspect, among the various new technologies developed, GM technology offers significant profits to farmers as they can reduce the present challenges in commercial agriculture and the current market forecast them as one of the world's rapidly flourishing creative sectors, benefiting not only farmers, customers and also contribute major economies in different countries. Currently, the new transgenic technologies like RNA interference-mediated gene silencing technology, gene targeting for enhanced efficiency and zinc-finger nuclease gene targeting technology are concentrating on finding novel genes and developing new approaches for plant biology research. Although, genetically modified crops are not only the universal solution to combat the problems of malnutrition and hunger, but also GM crops can act as an essential part of food safety programme. Thus, through new advances in gene integration techniques, in the development of stress resistance and biofortification, GM crops are expected to add efficiency and profit for commercial agriculture in future.

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Authors' contributions

All authors contributed equally in the preparation as well as revision of the manuscript and approved the final version.

Conflict of interest

The authors declare that they have no competing interests.

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