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Chapter 1

Introductory Chapter: Transgenics—Crops Tailored for Novel Traits

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1. Introduction

Transgenic crops are referred to as the genetically engineered crops. Traits, otherwise impossible to introduce by conventional approaches, are tailored using genetic manipulations and transformation approaches. Among traits is the introduction of agronomic, pathological, entomological, nutritional, therapeutic-, and vaccine-related characters in plants. The chapter covers state-of-the-art advancements in this rapidly developing area of transgenic technology and the technology for the food and health security mainly of poor populace in the developing countries.

This era has seen an explosive growth in population and urbanization, leading to an immense loss of agricultural land; therefore, the food security, especially which of poor populace, is of foremost importance. According to an estimate, this requires approximately 70% increment in food production by 2050. Since the 1990s, the introduction of insect resistance and herbicide tolerance into transgenic crops has increased the yield tremendously, benefiting farmers worldwide. Though production is increased by addressing problems of yield losses using transgenic technology, malnutrition is still one of the biggest challenges, demanding fortification of grains. Since nutrition is one of the main factors in maintaining a healthy lifestyle and meeting requirements of food security, several national nutrition surveys conducted in various countries have provided an avenue for governments to assess malnutrition problems across populations. Micronutrient deficiencies have been termed as the cause of "hidden hunger." Iron-fortified products are the prime examples of it. Pyramiding genes that encode provitamin A, transgenically or naturally, in crops like rice [1], potatoes [2], and maize [3, 4] have made these crops a rich source of provitamin A. In addition to adding nutritional elements in crops, the transgenic technology has led the scientists to tailor medicinal traits, for example, therapeutic [5, 6] and antigen proteins [7].

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Transgenic crops are developed in routine to express agricultural and medicinal traits, but it is very important to discuss the technologies used to develop them. Nuclear transformation is more successful in tailoring agricultural traits in crops (**Figure 1**), though it remained unfruitful in few genotypes like upland cotton in the Indo-Pak subcontinent, the reason being these genotypes are recalcitrant to regeneration from single cell, despite several crosses were made between genotypes to improve the regeneration potential, while chloroplast transformation is distinctly effective to tailor medicinal traits (**Figure 2**) [5, 6], the reason being nutraceutical, pharmaceutical, and antigenic proteins are required to be accumulated in exceptionally high amounts with bona fide structures. Chloroplasts are polyploid at organellar and genome levels and provide natural gene containment [8]; hence, they are preferred

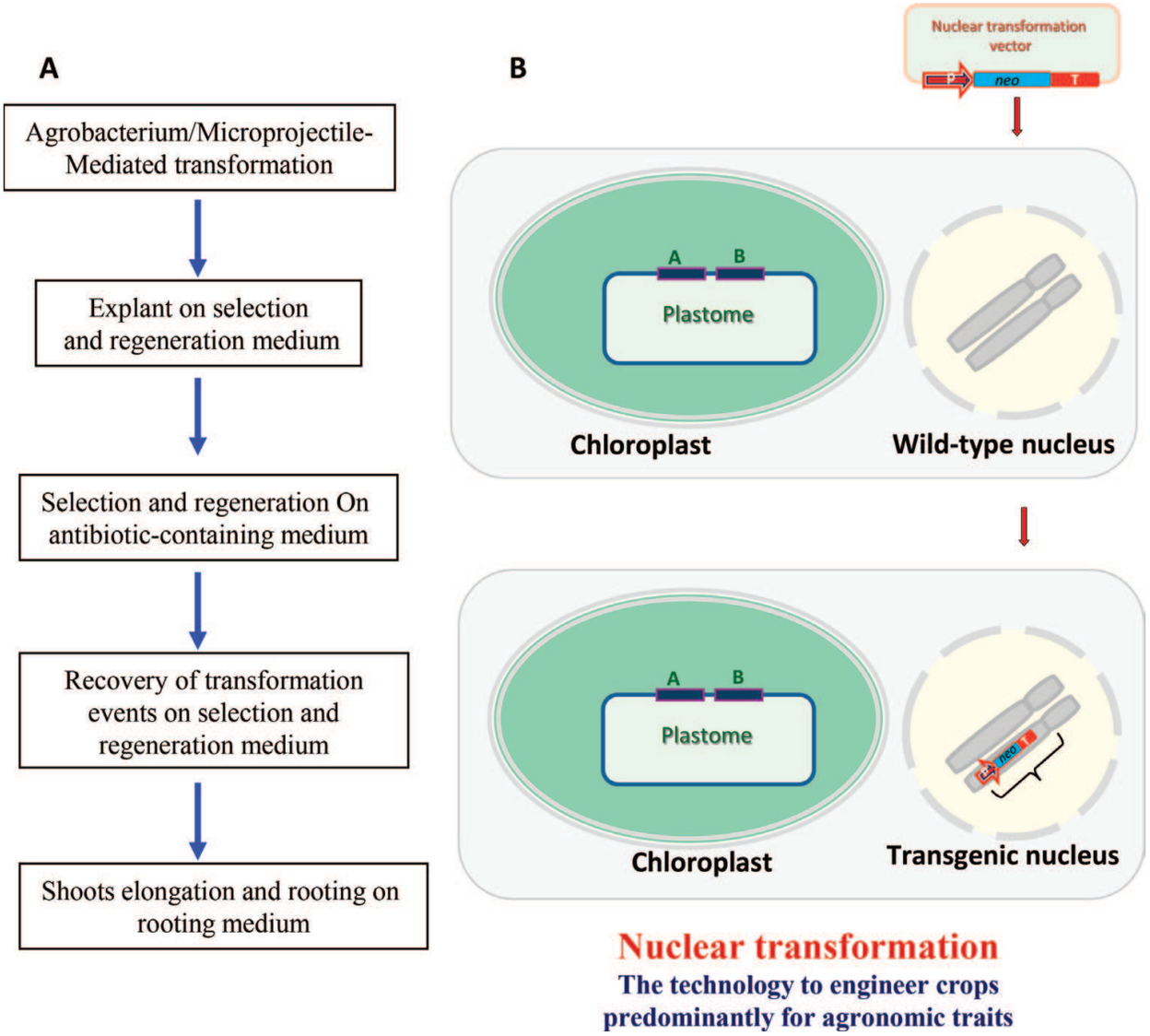

Figure 1. Transgenics predominantly for agronomic traits. Panel A explains the transformation steps involved in the development of transgenics. Panel B shows integration of transgene into the nuclear genome of crops via *Agrobacterium*-mediated or ballistic transformation approaches where transformation vector is either bombarded or cloned between left and right borders of a plasmid vector and multiplication using suitable *Agrobacterium* strain.

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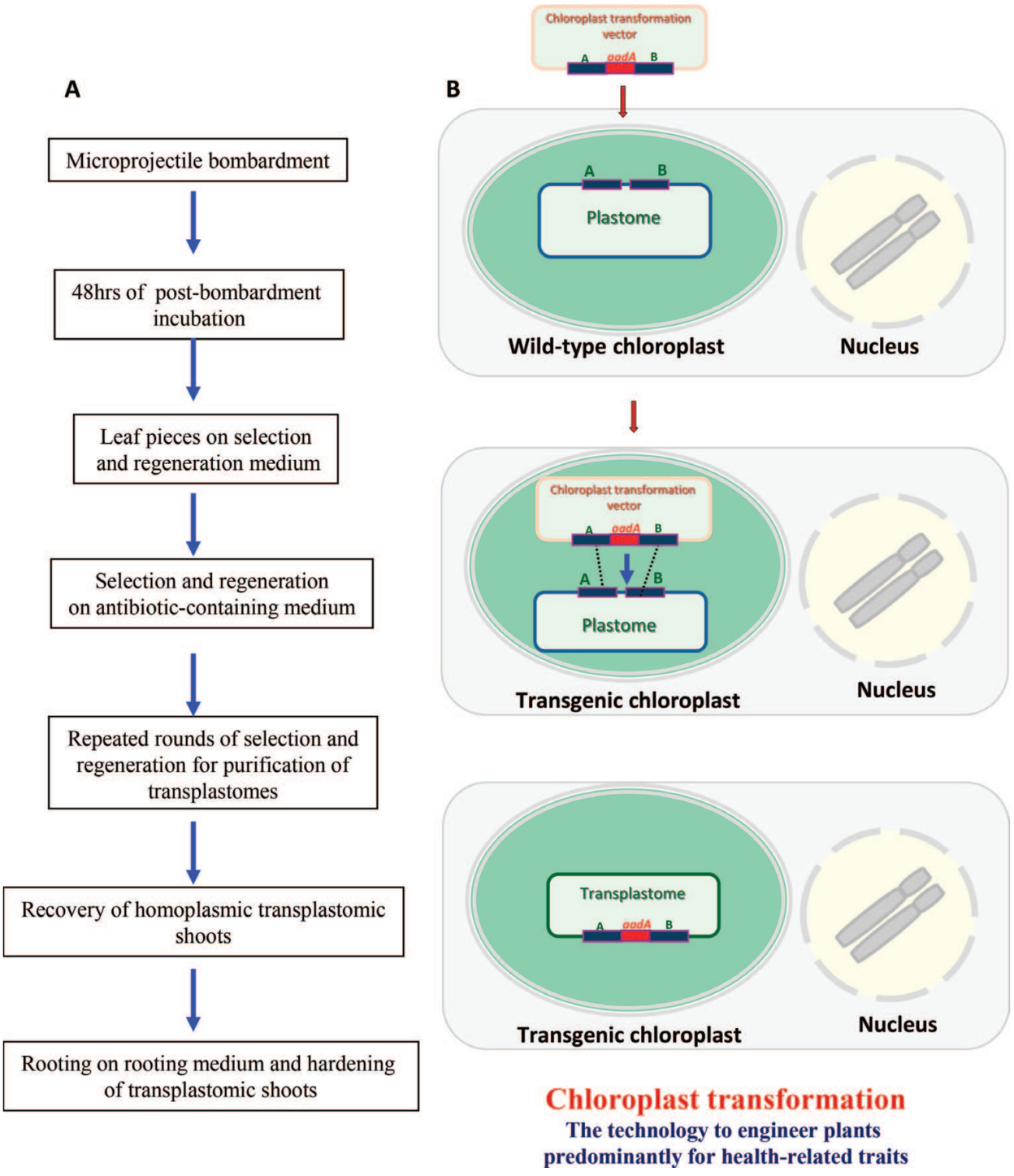

Figure 2. Transgenics predominantly for health-related traits. Panel A explains main steps involved in the development of transgenic chloroplasts to express transgenes that encode novel proteins to be used as nutraceuticals, therapeutics or vaccines. Panel B explains how a transgene from a transformation vector is integrated into the plastome via homologous recombination events to accumulate proteins to high levels with *bona fide* structures.

to express health-related traits rather agricultural. Plastid transformation though is achieved in wheat [9], rice [10], and sugarcane, but it is reproducible only in rice [11], yet transgenic plants remain heteroplasmic.

2. Developing transgenics: state-of-the-art strategies

A plant cell is blessed with three major organelles with their own genomes, namely, nucleus, chloroplast, and mitochondria. Of these three, two genomes are routinely manipulated to incorporate new traits in cultivated plants. There are a number of approaches to transfer and introduce genes into the plant genome, depending upon the choice of explant to be used in transformation experiments, for example, *Agrobacterium*-mediated gene transfer, gene gun, agro-infiltration, sonication, and polyethylene glycol treatment. Of these, *Agrobacterium*-mediated and gene gun methods are most commonly used approaches to develop transgenic plants. For nuclear transformation, *Agrobacterium* method is more successful than particle bombardment as a more number of transformed shoots can be recovered from the same number of explant.

Genetic transformation process involves a number of steps, including selection of a gene that confers resistance to a particular antibiotic for selection and screening purposes, isolation of a trait-encoding gene, choice of promoters and terminating sequences to control the expression of the gene or genes, choice of explant, and an artificial medium to support explant to regenerate into a complete shoot. For selection and screening, usually two types of markers are used: (1) selectable marker and (2) visual marker [10, 12–14]. Selectable marker could be lethal or nonlethal in nature. Nuclear genome transformation is carried out using lethal markers. Regenerated shoots are normally hemi- or heterozygous and need either further purification of transgenome using selection medium or through selfing depending upon the crop used.

The second genome is the chloroplast genome, the plastome that has been modified in a number of plant species, including model, crop, and tree plants. Plastome is a double-stranded DNA molecule of 152 (*Cinnamomum campohra*) – 218 kb (*Pelargonium*) size [15, 16]. Approximately, 120 genes in various plant species are encoded by the plastome [17]. It looks like that most of the ancestral genes have either been lost during evolution or transferred to the nucleus. A mature mesophyll cell contains up to 100 chloroplasts, and each chloroplast contains 100 plastome copies; therefore, the ploidy number of plastome per cell reaches up to 10,000 copies. Furthermore, this number is doubled for genes that are located in inverted repeat regions of the plastome [17].

When transforming a chloroplast, a universal antibiotic cannot be used given that different plants have variable sensitivity to selective agents; therefore, recovery of the transplastomic shoots is dependent on two things: (1) choice of the selective agent to be used and (2) the concentration of the selective agent that allows regeneration and development of shoots from the transformed cells while killing the non-transformed cells. For example, spectinomycin is used to select transformed cells on selection medium from tobacco, lettuce, tomato, potato, cabbage, oil rape seed, and carrot. However, several monocots, including rice and sugarcane, are naturally resistant to spectinomycin; therefore, streptomycin-containing medium was used for carrying out selection for transplastomic lines.

Initially, only few copies of the plastid genome are transformed and maintained under continuous selection pressure. However, stable lines with uniformly transformed genome copies are recovered on selection medium through a repeated cycle of regeneration [6]. During the period, the wild-type and the transgenic plastids and their genomes gradually sort out;

thereby, chimeric sectors, carrying wild-type or transgenic plastids, appear in leaves of regenerated shoots. Due to phenotypic masking by the transformed cells, both transgenic and wild-type cells in a chimeric shoot look green in color [6], indicating that antibiotic resistance is not cell autonomous. However, both wild-type and transformed sectors are identifiable using green fluorescent protein (GFP). This visual marker allows visual detection of the fluorescing transproteins because they produce green fluorescence upon illumination with blue or ultraviolet (UV) light [18, 6].

3. Transgenics for agricultural traits

3.1. Transgenics to improve crop production

Plants on earth synthesize their food by themselves, owing to harbor solar energy conversion and several other chemical reactions in their cells. One of the reactions carried out in plants is carbon fixation during a process, namely, photosynthesis. During photosynthesis Rubisco catalyzes the inefficient carbon fixation, reviewed extensively elsewhere [19, 20]. This raises a question why carbon fixation during photosynthesis is rate limiting. Major reasons are as follows: first, Rubisco's rate of catalysis is much low, and, second, it has to compete with a nonproductive reaction, oxygenation [21], depending upon the relative concentration of carbon dioxide and oxygen, as well as on temperature. Carboxylation results in CO_2 fixation. Therefore, plant growth and yield can be improved by two ways: (1) by increasing photosynthesis and (2) by reducing photorespiration.

A number of examples are available in the literature, reporting different versions of Rubisco that would improve photosynthesis [22], but considerable success has not been achieved yet. Introducing C_4 pathway in C_3 plants appears to be more promising, but due to the leakage of gases, the advantages of concentrating carbon dioxide in the chloroplasts of C_3 plants are objectionable [23]. Glycolate catabolic pathway was introduced in chloroplasts for alleviation of photorespiratory losses in *Arabidopsis thaliana*. Photosynthesis is markedly increased in this engineered pathway, thereby widening the applicability of the technique to cereals, for example, wheat and rice, described in detail elsewhere [24].

Developing chlorophyll in the dark, and chloroplasts that are competent for photosynthesis upon exposure to light, is another promising technique that can be implied to improve the photosynthesis in plants [25, 26]. In a study, *chlB* gene from *Pinus thunbergii* was introduced into the plastome of *Nicotiana tabacum* [27]. Transgenic plants when shifted to light from dark in early development of chlorophyll pigments were observed in leaves of transgenic compared to wild-type plants. This helps us to understand the molecular biology of transgenic angiosperms. Another effort is underway to introduce C_4 pathway in rice, a C_3 plant [28], using various techniques.

3.2. Transgenics for weed management

Weeds compete with crops for food, thereby lowering the crop yield and affecting farmers. There are two types of herbicides, (1) selective in nature and (2) used before and after emergence of plants from seeds, and are specific to leaf morphology.

A pioneering concept to engineer a crop for herbicide tolerance was developed in the 1980s [29] when it was observed that few herbicides kill plants by blocking photosynthetic electron transport. For example, triazine herbicides bind to a photosystem-II protein (D1) in the chloroplasts that appeared to be the first molecular target to develop a commercial herbicide [30]. However, tolerance to herbicides in transgenic plants is considered the best approach in weed control in crops. Glyphosate is a nonselective broad-spectrum herbicide that kills narrow-leaf grasses and broad-leaf weeds. Glyphosate competitively inhibits 5-enol-pyruvyl shikimate-3-phosphate synthase (EPSP) in the amino acid biosynthetic pathway. This proves to be a standard strategy to overcome the problem of herbicide selectivity. Yet, this strategy raises the concern of gene transfer to other plants or weeds.

An antibiotic bialaphos inhibits glutamine synthetase (GS) upon removal of alanine residues in the nitrogen assimilation pathway; resultantly, accumulation of toxic levels of ammonia in both bacteria and plant cells occurs. This antibiotic was used as an herbicide that appeared to be nonselective in nature. In two different studies, transgenic tobacco plants exhibited field-level tolerance to phosphinothricin (PPT) when bar was expressing from chloroplast genome [31, 32]. Further, development of glufosinate-resistant traits has been reported worldwide in corn, soybean, and cotton until now. The trait has also been developed by Khan and his team in sugarcane, and the transgenic plants were tolerant to BASTA [33]. The extensive and continuous use of a single herbicide should be avoided to exclude the possibilities of resistance development in plants, and precautionary measures should be taken to safeguard human health.

3.3. Transgenics for insect resistance

Engineering plant genomes for useful traits leads toward sustainable agriculture. Among useful traits, resistance against insects is developed by using Cry proteins from *Bacillus thuringiensis* (*Bt*). *Bacillus thuringiensis* is a soilborne bacterium, having crystal (Cry) proteins in the cytoplasm of the cells at sporulating stage. These proteins are toxic to some chewing and sucking insects.

Genes encoding Cry proteins have been expressed in a number of crops worldwide to control major pests. This has reduced the pesticide usage and has lowered the production costs of crops. First, transgenic crops developed were corn and cotton that expressed *cry1Ab* and *cry1Ac* genes, respectively [34]. Afterward, other crops including soybean, maize, cotton, canola, squash, papaya, tomato, sugar beet, and sugarcane were transformed using *Bt* genes to control insects. Almost all global biotech crop area is because of soybean, corn, cotton, and canola crops [35]. Since the first commercial cultivation of GM crops in 1996, farmers (16.7 million) from 29 countries cultivated 160 million hectares of biotech crops in 2011. Out of this number, about 90% were small and resource poor farmers belonging to developing countries. The United States and Brazil were major producers who adopted GM crops.

In Pakistan, first indigenously developed transgenic crop was sugarcane, carrying *cry1Ab* gene that was approved by the Technical Advisory and National Biosafety Committees after the approval of biosafety rules and guidelines in 2005. Developed sugarcane plants carry

Bt toxin only in green tops with no residues in the juice [36]. Lately, a different version of the *cry1Ab* gene was again used to develop transgenic sugarcane, and similar results were obtained.

3.4. Transgenics for pathogen resistance

Plant pathogens are damaging plants and causing yield losses exceptionally; it is therefore highly desirable to develop transgenics that would be resistant to pathogenic bacteria and fungi. There are a number of examples available in literature where pathogens have been targeted to control diseases in plants. Arrieta and colleagues in 1996 reported co-expression of genes encoding glucanase- and thaumitin-like proteins, and a low level of fungal infection was observed [37]. In other studies when snakin-1 gene was overexpressed transgenically, an enhanced resistance to *Rhizoctonia solani* and *Erwinia carotovora* was observed. Similarly, chitinase gene from *Streptomyces griseus* showed resistance against *Alternaria solani*, while expression of mycoparasitic chitinase and glucanase enzymes developed improved resistance to *Rhizoctonia solani*. Five novel thionin genes were isolated from plants belonging to the *Brassicaceae* family, and when expressed transgenically in potato, a high-degree resistance to gray mold (*Botrytis cinerea*) was observed [38]. Literature review suggests that broad-spectrum resistance could be attained in valuable plant species through transgenic technology. Mycoparasites can be controlled using glucanases, chitinases, proteases, cellulases, kinases, and certain antibiotics.

Amphipathic peptides such as magainin are known to control microbe infections; Daniell and his colleagues expressed MSI-99 in chloroplasts of tobacco and reported a varied degree resistance to microbes [39] with no changes in growth and development of the transgenic plants compared to wild-type plants. But using such genes in crops warrants extensive biosafety studies.

4. Transgenics for medicinal traits

4.1. Transgenics for nutraceuticals

One of the items on the wish list of biotechnologists is to engineer genomes of plants to tailor high-value traits other than agronomic, pathological, and entomological in nature. Among high-value traits are the introduction of nutrition and related characters. “Nutraceuticals” is a portmanteau of “nutrition” and “pharmaceuticals”; hence, the word implies that nutraceuticals are products regulated as medicine, food ingredients, and dietary supplements. These products not only provide protection against various diseases caused due to the deficiency of the nutrients but also have physiological benefits. Traditionally, nutraceuticals have been employed in the form of medicinal plants, etc., but in this modern era, nutraceuticals are being used in a variety of perspectives, such as nutrition and medicine. Iron-fortified products are the prime examples of it. Addition of iron-containing compounds during the grinding of wheat, otherwise deficient in iron, protects the wheat-dependant populace from diseases

caused by deficiency such as iron-deficiency anemias, etc. Iron fortification of wheat has been proven transgenically by expressing phytase gene (*phy*A) from *Aspergillus japonicas* [40]. In situ degradation of phytates in the seed endosperm is considered desirable in order to increase bioavailability of micronutrients [40].

Golden rice and provitamin A-fortified maize are crops that have caught interest of nutritionists globally. Provitamin A deficiency that results in night blindness in masses may be addressed through genetic improvement of crops like maize. In a study where single-cross maize yellow hybrids were evaluated for carotenoid contents [3] since biofortification of maize, endosperm is found to be the most convenient solution addressing its deficiency. Hence, improved contents of provitamin A carotenoids in maize may help Pakistani populace to alleviate the subclinical symptoms of vitamin A deficiency [4].

Perhaps, the most researched aspects of nutraceuticals are their use in medicine, to cure a variety of diseases such as cancer, osteoarthritis, cardiovascular disorders, etc. Over the years, several plants have been shown to contain compounds which, if incorporated into lifestyle early on, reduce the risk of cancer by as much as 33%. For example, blue maize has been found to be an effective nutraceutical in prevention of several types of cancers, such as colon cancer, etc. [41].

This era of rapid urbanization has seen an emerging trend of expressing many medicinal and nutritional traits into other food crops transgenically. Although several people have shown their concerns as to its biosafety, such drawbacks have not been reported to this date. Chloroplast transformation addresses biosafety issues as chloroplasts are not transmitted through pollens in most cultivated plants. Hence, the transgenics are a promising way forward to develop cost-effective nutraceuticals.

4.1.1. Transgenics for therapeutics

A number of pharmaceutical proteins have been synthesized exploiting plant genetic systems with overriding impact on conventional approaches used to manufacture pharmaceuticals. Some advantages of using plant system are low cost of production of pharmaceuticals and their processing. Commercial-scale production in bringing therapeutics to the clinic has been observed in the last 6–7 years. Manufacturing facilities of different capacities have been constructed in addition to the development of plant-made pharmaceuticals to meet current manufacturing standards [42]. Large Scale Biology Corporation (LSBC) in Owensboro, KY, USA, has designed the first manufacturing facility that plant virus transient expression system was developed to meet the current good manufacturing practice [43, 44].

However, synthesizing pharmaceuticals in plants by engineering the chloroplast genome is more advantageous as explained elsewhere in this chapter; therefore, attempts have been made to express different pharmaceuticals proteins. For example, interferons α2 and 5 were expressed from tobacco chloroplasts [5, 6]. In these studies the interferon α2 and 5 genes were synthesized and expressed. It was observed that fully expanded mature leaves contained high levels of interferon hen compared to young and senescence leaves; however, expression

levels were very low because of a mutation in the critical region of the promoter during synthesis. Other examples of therapeutic expression are human serum albumin (HSA) that was expressed between 0.02 and 11.1%, depending upon the regulatory sequences used [45], oral and injectable insulin [46], HIV inhibitor cyanovirin [47], TGFb3 [48], and thioredoxins from plastids as modulators of recombinant therapeutic protein production [49]. Recently, different companies and foundations like the Bill and Melinda Gates Foundation or Juvenile Diabetes Research Foundation are undertaking well to advance such developments from labs to the clinics.

4.2. Transgenics for antigenic proteins (vaccines)

Vaccination is an efficient strategy to control viral infections in both human and animal species. Different expression systems, having their own merits and demerits, are being used to produce recombinant vaccines. An ideal system would be that allows producing the desired functional product cost-effectively. Plant-based expression strategies encouraged biotechnologists to use this system to produce vaccines for both humans and animals. Moreover, plant system-derived subunit vaccines are heat stable, bio-encapsulated, and easy to scale up.

Oral vaccine term was introduced and extensively pursued after the successful expression of HBsAg in plants and recovery of the antigen as viruslike particles [50]. Interestingly, the antigen has the same properties as produced in yeast. Later on, the binding subunit of *E. coli* enterotoxin (LTB) and the capsid protein of norovirus genotype, which formed viruslike particles (NV-VLP), was expressed in plants that triggered mucosal immunization response in animals, hence, based on the data on approval clinical trials was obtained [7]. Diverse antigenic proteins were expressed in vegetable and fruit crops, and animal trials were successfully conducted. Plant species used are alfalfa, carrot, lettuce, tomato, potato, maize, soya bean, rice, and banana [51].

Development of an efficient plant-based system to express human antigenic proteins successfully has prompted its application to vaccinate livestock. Different attempts have been made by different research groups to address various diseases of livestock caused by viruses to increase the production in a cost-effective manner. Some of the examples are foot-and-mouth disease virus (FMDV), bovine rotavirus, bovine viral diarrhea virus, bluetongue virus, and bovine papillomavirus. Of these viruses, foot-and-mouth disease virus (FMDV) has been addressed majorly as livestock is an inevitable part of the economy. Livestock productivity is compromised due to frequent occurrence of foot-and-mouth disease (FMD). Vaccination is one of the main strategies to control foot-and-mouth disease. Yet, lack of high-quality and effective vaccine in Pakistan warrants the development of genotype-matched vaccines. One of the approaches to develop such vaccines is reverse genetics that is very costly and laborious. This demands exploring other alternative approaches. Of the alternative approaches, engineering edible plants with the pathogenicity-causing genes is more promising.

An oral vaccine against FMDV was developed by expressing structural protein VP1 in transgenic *Stylosanthes guianensis* [52]. In these experiments, the level of recombinant protein was

varied from 0.1 to 0.5% of total soluble protein. These levels were enough to induce a protective systemic antibody response in mice. In another attempt, capsid precursor polypeptide (P1) was expressed in rice, and 0.6–1.3 mg/g of TSP was observed that induced a protective immune response in mice [53]. Further, in mice when vaccinated orally, FMDV-specific mucosal immune responses were detected. However, partial virus clearance after challenge was observed. To address these low-expression problems, chloroplast transformation approach can be used. In a study, VP1 was expressed in tobacco chloroplasts and 2–3% values were recorded [54]. In another study, epitopes (B cell) of structural proteins VP1 and VP4 and of nonstructural proteins 2C and 3D (T cell) were produced in *N. benthamiana* plants using a plant virus expression system [55]. More recently, tandem-linked VP1 proteins of two serotypes, A and O, are expressed in forage crop *Crotalaria juncea* and fed to guinea pigs that produced humoral as well as cell-mediated immune responses [56]. From all these studies and experiments being carried out in the laboratory of the author of this chapter demonstrate that plant-based overexpression of antigenic proteins to control FMDV is an effective way but needs further experimentation to improve efficacy of edible vaccines by engineering epitopic proteins with different adjuvants (Khan MS, unpublished).

5. Conclusions

Nuclear transformation, achieved using microprojectile bombardment or *Agrobacterium* strains, is predominantly carried out to tailor agronomic traits in crops. However, this technology is not successful to transform upland cotton where cells are recalcitrant to regeneration, despite genome mixing through crosses between different genotypes. Biotechnologists rely on this technology though genes escape and pollinate other related crops or weeds, developing weeds or super weeds, respectively. An alternate strategy to develop transgenics is the chloroplast transformation technology since this technology offers natural gene containment, high-level transgene expression with bona fide structures of proteins, and allows all transformation events to be uniform as far as gene integration into the plastome is concerned. High-level gene expression is due to the polyploid nature of chloroplasts in a cell and of plastomes in each chloroplast, biologically active proteins are due to the presence of chaperon proteins, and uniform integration of transgenes into the plastome is due to the homologous recombination. Hence, chloroplast transformation is more suitable for expression of health-related traits in plants rather agronomic in crops. In either case, transgenics should be grown in the field following approved biosafety guidelines and strict stewardship.

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References

[1] Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, et al. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science. 2000;**287**:303-305

[2] Diretto G, Welsch R, Tavazza R, Mourgues F, Pizzichin D, Beyer P, et al. Silencing of beta-carotene hydroxylase increases total carotenoid and beta-carotene levels in potato tubers. BMC Plant Biology. 2007;**7**:11

[3] Maqbool MA, Aslam M, Khan MS, Beshir B, Ahsan M. Evaluation of single cross yellow maize hybrids for agronomic and carotenoid traits. International Journal of Agriculture and Biology. 2017;**19**:1087-1098

[4] Maqbool MA, Aslam M, Beshir A, Khan MS. Breeding for provitamin A biofortification of maize (*Zea mays L.*). Plant Breeding. 2018;**137**:451-469

[5] Nurjis F, Khan MS. Expression of recombinant interferon alpha-2a in tobacco chloroplasts using microprojectile bombardment. The African Journal of Biotechnology. 2011;**10**:17016-17022

[6] Khan MS, Nurjis F. Synthesis and expression of recombinant interferon alpha-5 gene in tobacco chloroplasts, a non-edible plant. Molecular Biology Reports. 2012;**39**:4391-4400

[7] Giorgi C, Franconi R, Rybicki EP. Human papillomavirus vaccines in plants. Expert Review of Vaccines. 2010;**9**:913-924

[8] Khan MS. Plastid genome engineering in plants: Present status and future trends. Molecular Plant Breeding. 2012;**3**:91-102

[9] Cui C, Song F, Tan Y, Zhou X, Zhao W, Ma F, et al. Stable chloroplast transformation of immature scutella and inflorescences in wheat (*Triticum aestivum L.*). Acta Biochimica et Biophysica Sinica. 2011;**43**:284-291

[10] Khan MS, Maliga P. Fluorescent antibiotic resistance marker to track plastid transformation in higher plants. Nature Biotechnology. 1999;**17**:910-915

[11] Lee SM, Kang K, Chung H, Yoo SH, Xu XM, Lee SB, et al. Plastid transformation in the monocotyledonous cereal crop, rice (*Oryza sativa*) and transmission of transgenes to their progeny. Molecular Cell. 2006;**21**:401-410

[12] Khan MS. Tobacco chloroplast transformation using microprojectile bombardment [PhD Diss.]. UK: University of Cambridge; 1997

[13] Hibberd JM, Linley PJ, Khan MS, Gray JC. Transient expression of green fluorescent protein in various plastid types following microprojectile bombardment. The Plant Journal. 1998;**16**:627-632

[14] Khan MS. Utilizing heterologous promoters to express green fluorescent protein from jellyfish in tobacco chloroplasts. Pakistan Journal of Botany. 2001;**33**:43-52

[15] Chen C, Zheng Y, Liu S, Zhong Y, Wu Y, Li J, et al. The complete chloroplast genome of *Cinnamomum camphora* and its comparison with related Lauraceae species. Peer Journal. 2017;**5**:e3820. DOI: 10.7717/peerj.3820

[16] Chumley TW, Palmer JD, Mower JP, Fourcade HM, Calie PJ, Boore JL, et al. The complete chloroplast genome sequence of Pelargonium x hortorum: Organization and evolution of the largest and most highly rearranged chloroplast genome of land plants. Molecular Biology and Evolution. 2006;**23**:2175-2190

[17] Sugiura M. The chloroplast genome. Plant Molecular Biology. 1992;**19**:149-168

[18] Khan MS, Zafar Y, Malik KA. Light that reflects your effort. International Journal of Agriculture and Biology. 2000;**2**:396-399

[19] Cleland WW, Andrews TJ, Gutteridge S, Hartman FC, Lorimer GH. Mechanism of *Rubisco*: The carbamate as general base. Chemical Reviews. 1998;**98**:549-561

[20] Bock R, Khan MS. Taming plastids for a green future. Trends in Biotechnology. 2004;**22**:311-318

[21] Wingler A, Lea PJ, Quick WP, Leegood RC. Photorespiration: Metabolic pathways and their role in stress protection, philos. Philosophical Transactions of the Royal Society B. 2000;**355**:1517-1529

[22] Lin MT, Occhialini A, Andralojc PJ, Parry MA, Hanson MR. A faster *Rubisco* with potential to increase photosynthesis in crops. Nature. 2014;**513**:547-550

[23] Tolbert NE. The C_2 oxidative photosynthetic carbon cycle. Annual Review of Plant Physiology and Plant Molecular Biology. 1997;**48**:1-25

[24] Kebeish R, Niessen M, Thiruveedh K, Bari R, Hirsch H-J, Rosenkranz R, et al. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. Nature Biotechnology. 2007;**25**:593-599

[25] Khan MS. Engineering photorespiration in chloroplasts: A novel strategy for increasing biomass production. Trends in Biotechnology. 2007;**25**:437-440

[26] Yamamoto H, Kurumiya S, Ohashi R, Fujita Y. Oxygen sensitivity of a nitrogenase-like protochlorophyllide reductase from the cyanobacterium *Leptolyngbya boryana*. Plant and Cell Physiology. 2009;**50**:1663-1673

[27] Yamazaki S, Nomata J, Fujita Y. Differential operation of dual protochlorophyllide reductases for chlorophyll biosynthesis in response to environmental oxygen levels in the cyanobacterium *Leptolyngbya boryana*. Plant Physiology. 2006;**142**:911-922

[28] Nazir S, Khan MS. Chloroplast-encoded *chlB* gene from *Pinus thunbergii* promotes root and chlorophyll pigment development in *Nicotiana tabaccum*. Molecular Biology Reports. 2012;**39**:10637-10646

[29] Hibberd JM, Sheehy JE, Langdale JA. Using C_4 photosynthesis to increase the yield of rice–Rationale and feasibility. Current Opinion in Plant Biology. 2008;**11**:228-231

[30] Steinback KE, McIntosh L, Bogorad L, Arntzen CJ. Identification of the triazine receptor protein as a chloroplast gene product. Proceedings of the National Academy of Sciences of the United States of America. 1981;**78**:7463-7467

[31] Fuerst EP, Norman M. Interactions of herbicides with photosynthetic electron transport. Weed Science. 1991;**39**:458-464

[32] Lutz KA, Knapp JE, Maliga P. Expression of bar in the plastid genome confers herbicide resistance. Plant Physiology. 2001;**125**:1585-1590

[33] Kang T-J, Seo J-E, Yang MS. Herbicide resistance of tobacco chloroplasts expressing the bar gene. Molecular Cell. 2003;**16**:60-66

[34] Khan MS, Ali S, Iqbal J. Developmental and photosynthetic regulation of *Bacillus thuringiensis* δ-endotoxin reveals that engineered sugarcane conferring resistance to 'dead heart' contains no toxins in cane juice. Molecular Biology Reports. 2011;**38**:2359-2369

[35] Tabashnik BE, Unnithan GC, Massonb L, Crowder DW, Lia X, Carrierea V. Asymmetrical cross-resistance between bacillus thuringiensis toxins *Cry1Ac* and *Cry2Ab* in pink bollworm. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:11889-11894

[36] Brookes G, Barfoot P. Global impact of biotech crops: Environmental effects, 1996-2010. GM Crops and Food: Biotechnology in Agriculture and the Food Chain. 2012;**3**:129-137

[37] Arrieta JG, Enriquez GA, Suarez V, Estevez A, Fernandez ME, Menedez C, et al. Transgenic potato expressing two antifungal proteins. Biotecnologia Aplicada. 1996;**13**:121

[38] Hoshikawa K, Ishihara G, Takahashi H, Nakamura I. Enhanced resistance to gray mold (*Botrytis cinerea*) in transgenic potato plants expressing thionin genes isolated from *Brassicaceae* species. Plant Biotechnology. 2012;**29**:87-93

[39] De Gray G, Rajasekaran K, Smith F, Sanford J, Daniell H. Expression of an antimicrobial peptide via the chloroplast genome to control phytopathogenic bacteria and fungi. Plant Physiology. 2001;**127**:852-862

[40] Abid N, Khatoon A, Maqbool A, Irfan M, Bashir A, Asif I, et al. Transgenic expression of phytase in wheat endosperm increases bioavailability of iron and zinc in grains. Transgenic Research. 2017;**26**:109-112

[41] Guzmán-Gerónimo RI, Alarcón-Zavaleta TM, Oliart-Ros RM, MezaAlvarado JE, Herrera-Meza S, Chávez-Servia JL. Blue maize extract improves blood pressure, lipid profiles, and adipose tissue in high-sucrose diet induced metabolic syndrome in rats. Journal of Medicinal Food. 2017;**20**:110-115

[42] Wirz H, Sauer-Budge AF, Briggs J, Sharpe A, Shu S, Sharon A. Automated production of plant-based vaccines and pharmaceuticals. Journal of Laboratory Automation. 2012;**17**:449-457

[43] Pogue GP, Lindbo JA, Garger SJ, Fitzmaurice WP. Making an ally from an enemy: Plant virology and the new agriculture. Annual Review of Phytopathology. 2002;**40**:45-74

[44] Pogue GP, Vojdani F, Palmer KE, Hiatt E, Hume S, Phelps J, et al. Production of pharmaceutical-grade recombinant aprotinin and a monoclonal antibody product using plant-based transient expression systems. Plant Biotechnology Journal. 2010;**8**:638-654

[45] Millán AF-S, Mingo-Castel A, Miller M, Daniell H. A chloroplast transgenic approach to hyper-express and purify human serum albumin, a protein highly susceptible to proteolytic degradation. Plant Biotechnology Journal. 2003;**1**:71-79

[46] Boyhan D, Daniell H. Low-cost production of proinsulin in tobacco and lettuce chloroplasts for injectable or oral delivery of functional insulin and C-peptide. Plant Biotechnology Journal. 2011;**9**:585-598

[47] Elghabi Z, Karcher D, Zhou F, Ruf S, Bock R. Optimization of the expression of the HIV fusion inhibitor cyanovirin-N from the tobacco plastid genome. Plant Biotechnology Journal. 2011;**9**:599-608

[48] Gisby M, Mellors P, Madesis P, Ellin M, Laverty H, O'Kane S, et al. A synthetic gene increases TGFβ3 accumulation by 75-fold in tobacco chloroplasts enabling rapid purification and folding into a biologically active molecule. Plant Biotechnology Journal. 2011;**9**:618-628

[49] Sanz-Barrio R, Millán AF-S, Corral-Martínez P, Seguí-Simarro JM, Farran I. Tobacco plastidial thioredoxins as modulators of recombinant protein production in transgenic chloroplasts. Plant Biotechnology Journal. 2011;**9**:639-650

[50] Mason HS, Lam DM, Arntzen CJ. Expression of hepatitis B surface antigen in transgenic plants. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**:11745-11749

[51] Ahmad P, Ashraf M, Younis M, Hu X, Kumar A, Akram NA, et al. Role of transgenic plants in agriculture and biopharming. Biotechnology Advances. 2012;**30**:524-540

[52] Wang DM, Zhu JB, Peng M, Zhou P. Induction of a protective antibody response to FMDV in mice following oral immunization with transgenic *Stylosanthes spp.* as a feed-stuff additive. Transgenic Research. 2008;**17**:1163-1170

[53] Wang Y, Shen Q, Jiang Y, Song Y, Fang L, Xiao S, et al. Immunogenicity of foot-and-mouth disease virus structural polyprotein P1 expressed in transgenic rice. Journal of Virological Methods. 2012;**181**:12-17

[54] Li Y, Sun M, Liu J, Yang Z, Zhang Z, Shen G. High expression of foot-and-mouth disease virus structural protein VP1 in tobacco chloroplasts. Plant Cell Reports. 2006;**25**:329-333

[55] Andrianova EP, Krementsugskaia SR, Lugovskaia NN, Mayorova TK, Borisov VV, Eldarov MA, et al. Foot and mouth disease virus polyepitope protein produced in bacteria and plants induces protective immunity in guinea pigs. Biochemistry (Moscow). 2011;**76**:339-346

[56] Rao JP, Agrawal P, Mohammad R, Rao SK, Reddy GR, Dechamma HJ, et al. Expression of VP1 protein of serotype A and O of foot-and-mouth disease virus in transgenic sunnhemp plants and its immunogenicity for guinea pigs. Acta Virologica. 2012;**56**:91-99

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