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# A REVIEW OF GENE CLONING AND EDITING STRATEGIES FOR PHOTOSYNTHESIS IMPROVEMENT

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**Abstract:**

Photosynthesis seems to be a central process which converts the sunlight to energy, thereby promotes plant proliferation and development, and leads to the maintenance of life on the earth by using water to introduce oxygen into the environment. Increased plant photosynthesis has recently been revitalized as a strategy to increase yields of crops and solve global food problems. Photosynthesis, thus, has become a primary focus of genetic engineering. Multiple attempts are ongoing to improve photosynthesis in order to overcome the obstacles of developing food and fuel demand in speedily evolving global environments. In order to improve photosynthetic capacity, several transgenes have been inserted into the plastid or nuclear genomes. Plant transformation has become a key experimental approach in plant science as well as a functional approach for transgenic plant growth. There are several confirmed strategies for the effective integration of desired genes into the genomes of various plants. This review article explains the importance of the genetic engineering in the modification and improvement of photosynthesis and its potential utilization in increasing crop yields.

**Keywords** Photosynthesis, genetic engineering, transgenes, plant transformation, transgenic plant.

## INTRODUCTION

Photosynthesis is an essential system which controls the rate of Carbon dioxide fixing in plants and, eventually, the amount of carbohydrates employed in producing woody tissue in trees [1]. Photosynthesis comprises a set of biochemical pathways in which plants utilize sunlight to convert environmental Carbon dioxide to carbohydrates and release Oxygen as a secondary product. The oldest photosynthetic species, *Archean eon*, Single-celled ocean-dwelling prokaryotes originally appeared 2.5 billion years ago. As a result, photosynthesis was initially an underwater system that occurred in a highly reducing environment [2]. Consequently, the move to a planetary atmosphere and an oxidizing environment developed the photosynthetic process into its present shape [3]. Plant cells possess chloroplasts, organelles produced from a cyanobacterial-like organism's endosymbiosis. The photosynthetic systems that give plants its color are found in chloroplasts. Chlorophyll and other supplementary pigments absorb visible light from the sun, which is subsequently used to stimulate the electrons of the water molecule in the chloroplast's thylakoid membrane. Elevated electrons will then be passed to the carrier molecules, by which they can be donated in the chloroplast stroma for reducing the gaseous carbon dioxide to triose-phosphates. The enzyme Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenaseis) required for the carbon dioxide fixing first step. The ribulose-1,5-bisphosphate molecule regenerates due to the Calvin-Benson cycle [4], while the Carbon dioxide fixed passes into the pathways of anabolic for starch and sucrose biosynthesis [5]. Nowadays, there is a tremendous need for biotechnology and plant engineering to enhance crop yields for a variety of purposes. Amongst them, the environmental change, the raise of the human population, the depletion of farm land because of urbanization, soil destruction and the increasing demand for food crops as sources of energy must be regarded [6]. Increasing our interpretation of the molecular mechanisms of photosynthesis is important for the improvement of plant growth and development of the biomass. Modern investigations have demonstrated that genetic alteration of the genes participating in the photosynthetic process could modify the photosynthetic effectiveness and also

the plant productivity [7]. Plant genetic engineering allows the direct incorporation of the beneficial genes into the plants and provides an essential method for breeding projects by generating new and genetically distinctive plant products. The intended beneficial gene transition from certain organism to the other and the resulting integration that is stable and translation the foreign gene into the genome is termed that as "Genetic Transformation". The gene transform is identified ''transgene'', and organism which grows after effective gene transformation is defined as ''transgenic'' [8]. The present article discusses the importance of photosynthesis in the energy production and the opportunities for engineering the photosynthetic systems to improve the productivity and effective generation of photosynthetic biomass.

## NATURAL PHOTOSYNTHESIS

Photosynthesis is found to be a crucial biological system that enables the organisms to convert the sunlight energy to the ATP and NADPH necessary for the fixation of carbon dioxide [9]. Such a system is performed by two distinct pathways: light-dependent pathway and light independent pathway. In light-dependent pathway, after the absorbance of photons by antenna chlorophyll systems resulting in the stimulation of particular chlorophyll pairs, water is split and charges are separated to generate ATP and NAD(P)H. ATP and NADPH are used in the light independent pathway to produce sugar and many organic compounds that are significant from $CO_2$ [10]. In the process of photosynthesis, two distinct categories of photosynthetic organisms are described depending on the oxygen production. The first category, like green bacteria, purple bacteria and heliobacteria, possesses just single photosystem and employs reduced inorganic chemicals as electron donors [11]. Other category, such as higher plants, algae, and cyanobacteria, has two photosystems and uses water as just an electron donor, and therefore produces molecular Oxygen [12]. Furthermore, according on the form of the photochemical reaction center, photosynthetic organisms are classified as type (I) reaction centers, which Iron sulfur is used as a final electron receptor, including green sulfur bacteria and heliobacteria, and type (II) reaction centers which utilize quinone as a final electron receptor, like the filamentous bacteria [13]. It must be mentioned that the higher plants, algae, and cyanobacteria contain both PSI and PSII forms; therefore, these species could perform oxygenic photosynthesis, whereas all other photosynthetic prokaryotes perform anoxygenic photosynthesis only [13].

## NATURAL ANTENNA SYSTEM

The first crucial stage in photosynthesis is the efficient capture of sunlight by antenna pigments. The Photosystem II (PS-II) crystal structure in higher plants and cyanobacteria has been determined in recent years, revealing the presence of PS-II in a dimeric shape [14]. Each monomeric cluster consists of (19-20) subunits of protein that carrying antenna pigment complement of approximately (35) chlorophyll a (Chl-a), (20) lipid molecules, (11) ß-carotenes, (4) manganese atoms, (3) or (4) calcium atoms (one of which is in the Mn4Ca cluster), (3) chloride (2) plastoquinones, (2) pheophytins, (2) hematic irons and (1) non-hematic iron, [14]. Although Photosystem I (PS-I) is found in monomeric type in algae and higher plants [15], it is typically found in trimeric type in cyanobacteria [16]. Each monomeric PS-I molecule consists (96) Chl-a, (22) ß-carotene, (3) of 4Fe4S complexes, (2) phylloquinone molecules, and (4) lipid molecules in the (12-14) protein subunits [17]. As the effectiveness of the photosynthetic pathway that is light-dependent is primarily dependent on light absorption, both algae and higher plants have exterior antenna systems which might capture and transport a a significant amount of sunlight to the reaction centers [14]. Higher plants possess light harvesting complex systems (LHC) which capture the sunlight and transport it to PS-I and PS-II complexes. The LHC-II antenna system has been widely used in PS-II of higher plants and Chlorophyta [18]. Each one possesses (14) molecules of chlorophyll which characterized as (8) Chl-a and (6) Chl-b molecules. All the molecules of Chl-b organized around neighboring monomers interface. Each monomer contained (4) carotenoid molecules. In addition to their absorption properties, xanthophyll molecules are thought to be important in non-radiation energy dissipation [18]. There have been four classes of light harvesting clusters that connect to PS-I side of the PS-I antenna system of chlorophyta and higher plants. Approximately (20) molecules of chlorophyll are organized in strategic positions between LHCa and the center. The function of such clusters is confirmed not only by the supply of the necessary sunlight energy, but also by the developmental influences which have formed the chloroplasts of planetary plants [19]. In Cyanobacteria, the X-ray analysis of the PS-II complex revealed the presence of the PS-II complex in a dimeric shape and each monomer contains (36) chlorophyll a and (7) molecules of ß-carotene [20]. On the other hand, PS-I is primarily found in a trimeric shape. Each monomer includes approximately 96 Chl-a molecules and 22 ß-carotene molecules [17]. In addition, Cyanobacteria include an external light harvesting complex system in comparison to those found in higher plants defined as phycobilins. Energy conversion on normal and mutant apophycobilins in Cyanobacteria has revealed that energy is delivered to both PS-I and PS-II almost instantly [21]. Phycobilins are proteins that are soluble in water contain dyes [22]. Phycobilins on the thylakoid membrane's surface allow them to distribute over short distances and, as a result, could regulate the spread of the relative energy transport from phycobilisomes to PS-II and PS-I. Employing photobleaching fluorescence recovery (FRAP), phycobilins are mobile protein complexes which diffused speedily on the thylakoid membrane surface depending on the incident energy, according to studies [22]. Through soaking cells of cyanobacterial in high osmotic pressure buffers, the phycobilins diffusion is prevented [23]. In such situations, cells are restricted in state 1 or 2 depending on the circumstances until they are immersed in buffers [23]. Time-resolved fluorescence research have shown that approximately 50–60% of phycobilins are separated from PS-II during the transfer to state 2, and energy

storage research have shown such phycobilins are practically bound to PS-I [24]. As a result, the relationship between phycobilisomes and reaction centers appears to be temporary and unstable. [22]. The transportation of phycobilins from PS-II to PS-I when moving from light to dark environments has been proposed. During the dark phase of incubation, Cyanobacteria adjust to state 2, where the antenna connects primarily to PS-I, whereas at constant light system, Cyanobacteria adjust to state 1 and the antenna connects primarily to PS-II [25].

## TRANSFORMATION SYSTEMS

Plant modification has become a key approach in the research of plant biology and a functional approach for genetically modified plant growth. There are several documented strategies for the stable insertion of new genes into the genomes of various plants. In tobacco, the possibility to insert and express various foreign genes was first mentioned in 1984 [26], which has then been developed to include several plants of more than 35 families. Genetic modification achievements involve most of the significant economic crops, vegetables and medicinal plants. Consequently, inserting gene and regenerating transgenic plants have no more caused limitation of the production and application of functional transformation strategies for several plants. Strategies have evolved to resolve a wide range of obstacles encountered in the initial stages of plant transformation advancement. Gene transfer technologies require the utilization of many strategies to deliver isolated foreign genes to the host cell. There are currently two categories of transfer methods, namely non-biological methods and biological methods. The need for better transformation effectiveness has provoked research not only to develop the multiple current techniques, but also to invent new techniques [27].

### 1. Non-biological based transformation

- **Biolistics**

Particle bombardment, which had first been identified as an approach for the formation of genetically modified plants in 1987 [28], was described as an alternate to the protoplast transformation and, in particular, to the transformation of more recalcitrant cereals. The distinctive benefits for such approach opposed to different modes of propulsion approaches are explained elsewhere in respect of species and genotypes selection which transformed and high frequency for significant crops transformation [29]. In plant science, the main uses of biolistics are experiments on transferred gene expression, the generation of genetically modified plants and viral pathogens inoculation [30]. Biolistics gene designs could take the plasmids, either circular or linear, or a linear expression cassette. The best explants for biolistic transformation are probably embryonic cell cultures since they could be distributed as standardized target cells and have a high rate of recovery [31]. Rice transformation was also efficiently accomplished by the bombardment of embryonic calli [32], where transformation capacity can be improved to 50% [33]. Particle bombardment was developed as a reproducible approach for the transformation of wheat [34] and the earliest stable transformation of economically valuable conifer species, *Picea glauca*, has been accomplished through embryonic callus tissue as an explant [35]. Nevertheless, the bombardment of particles has certain limitations. The efficacy of transformation may be lower than with Agrobacterium-mediated transformation, and it is often more expensive. Moreover, this approach does not provide a protection to DNA against degradation, and its intracellular targets are random. As a consequence, several scientists ignored particle bombardment due to the higher occurrence of the complicated integration patterns and numerous copies inserts which might induce gene silencing and transgene expression variations [36].

### 2. Biological gene transfer

- **Transformation of *Agrobacterium* mediated**

The *Agrobacterium tumefaciens*, soil bacteria, and *Agrobacterium rhizogenus* natural capacity to transform host plants was used for transgenic plants generation. The possibility of employing A. Tumefaciens to deliver exogenous genes to plants logically was revolutionary in the 1970s. The plants genetic transformation has been seen as a promise. Agrobacterium had been rational and normal transformation candidate to be considered because it automatically delivers DNA (T-DNA) found on the tumor-inducing plasmid (Ti) to the plant cells nucleus and permanently integrates DNA into the genome of plant [37]. This approach has then become the most commonly employed and effective methodology for the development of genetically modified plants. Nevertheless, several obstacles are being encountered for the genotype-independent transformation of several commercially significant crop plants and also forest plants [38]. Despite the existence of other non-biological approaches to transform the plants [39], *Agrobacterium*-mediated transformation is common and is one of the most efficient approaches. That's true especially of many dicotyledonous plants, which are normally infected with Agrobacterium. Genes mediated by *Agrobacterium* delivery to plant of monocotyledonous, not considered too just be feasible. Nonetheless, seen the development of repeatable and effective approaches for rice [40], banana [41], corn [42], wheat [43], sugarcane [39], tall fescue (*Festuca arundinacea*) and forage grasses like Italian ryegrass (*Lolium multiflorum*) [44]. The first of the important commercial conifers to be irreversibly altered by co-cultivation of embryonic tissue with *A. Tumephacians* was hybrid larch [45]. And further, such approach was effectively extended to many spruce species [46]. Methodologies related to the transformation targets could even be divided into two classifications, namely involving in planta approaches and tissue culture. In tissue culture methods for plant transformation, a most significant necessity is a sufficient number of regenerable cells which are appropriate for gene delivery treatment and therefore can maintain regeneration potential for the period of the required target preparation, cell proliferation and selection treatments. The increased proliferation rate of the micropropagation method does not generally imply a

sufficient number of regenerable cells suitable for gene delivery [47]. Sometimes gene delivery into regenerable cells might not permit the recovery of transgenic plants because the ability for effective regeneration is short-lived [48]. Furthermore, approaches dependent on tissue culture could result in undesired somaclonal alterations, such as cytosine methylation variations, point mutation activation, and multiple chromosomal abnormalities [49]. For the other side, the realization of the all-transgenic plants was challenging in a great variety of crop plants, since those plants were shown to be extremely resistant in vitro. Consequently, other approach has been developed where the tissue culture element excluded from the process and this is defined as in planta strategy. Plant genetic engineering is beneficial to the molecular genetic research, crop enhancement and pharmaceutical products development. *Agrobacterium*-based approaches are commonly preferable to several organisms, like monocots and dicots. The others are not usually performed on a regular basis. Biolistics is perhaps the most commonly utilized direct transformation process, both experimentally and economically. Then why have those other approaches evolved in the last two or three decades, since we already have Agrobacterium and Biolistics transformation strategies? Two reasons are present. First, it is hoped that a much more effective and cheaper approach could be evolved. The next and most significant explanation is that the biolistics and *Agrobacterium* are invented [27].

- **In planta transformation**

Even though efficient plant regeneration techniques have become evolved, these techniques have never produced regeneration in many other plants to be used in transformation procedures, which severely restricts the utilization of gene delivery techniques to their highest capacity. In the presence of the great limitation, it is important to develop transformation methods which do not rely on the regeneration of tissue culture or others which significantly remove the involvement of tissue culture. In planta techniques offer this advantage. Strategies which require the transfer of transgenes in the shape of naked DNA directly to plants are referred to as in planta transformation strategies. These strategies avoid the steps of tissue culture, depend on basic procedures, and need a limited period of time to achieve whole transformed organisms. In certain situations, in planta strategies, meristems or other tissues have been targeted, assuming that when fertilized, the egg cell receives the transfer of the whole genome from the sperm cell, which would eventually result in zygotes [50] and would thus be the appropriate stage for the integration of transgenes. For in planta transformation methods which depend on non-tissue culture, *Agrobacterium* co-cultivation or microprojectile bombardment has been guided to transform cells in or around apical meristems [50]. Naked DNA insertion into ovaries was also documented to generate transformed offspring [51]. *Arabidopsis thaliana* has been the earliest plant which showed effective in planta transformation. The initial stages of development in the transformation of *Arabidopsis* emerged from the research of Feldmann and Marks [52]. Levels of transformation increased considerably when Bechtold et al. [53] inoculated plants that are in the flowering stage. Currently, There's only a few extremely organisms which could systematically transformed to lack of a regeneration method dependent on tissue culture. Arabidopsis could be transformed using a variety of in planta strategies, like vacuum infiltration [54], germinating seeds transformation [52] and flower dipping [54]. Other plants which have effectively experienced vacuum infiltration usually involve rapeseed, *Brassica campestris* and radish, *Raphanus sativus* [55]. The labor-intensive vacuum infiltration method has been removed in preference of a basic dipping of the growing floral tissue [54]. The research also revealed that the *Agrobacterium* floral spray system could generate higher levels of in planta transformation compared to vacuum infiltration and the floral dipping technique [56]. Cotton transformants were recovered using naked DNA after injecting DNA into the axil placenta a day after self-pollination. [51]. Likewise, either a combination of DNA and pollen has been added to receptive stigma surfaces, or DNA was inserted directly into rice flower tillers, or soybean seeds have been imbibed with DNA [57]. Such techniques, as interesting as they are, are currently inefficient due to their poor reproducibility. New investigations with *Agrobacterium* inoculation of germinating rice seeds have revealed transformation efficiency levels exceeding 40% [58], whereas producing 4.7 _ 76% efficiency levels for flower infiltration technique and 2.9 _ 27.6% efficiency level for the seedling infiltration process [59]. Crop plants which have effectively been transformed through injuring the apical meristem of the differentiated embryo of the germinating seeds, followed by infecting with *Agrobacterium*, are peanut, *Arachis hypogaea L.* [60], sunflower, *Helianthus annuus L.* [61], safflower, *Carthamus tinctorius L.* [62], field bean, *Dolichos lablab L.* [63], and cotton, *Gossypium sp.* [64]. The achievements mentioned before have indeed offered a high efficiency for easily developing transgenic plants, since the technique is simple, cost efficient, does not require a large infrastructural demand for the handling of recalcitrant plants like groundnuts. Therefore, gene delivery technology for the production of recalcitrant plants has become a reasonable approach for studying and generating successful transformants. Nevertheless, improving the association between *Agrobacterium* and the plant is essential for effective transformation. Multiple aspects, such as the kind of explant, are crucial and should be appropriate for the recovery of total transgenic plants [36]. Even though biotechnological developments have presented several techniques for gene delivery to plant cells, almost all transformation research totally depends on particle bombardment with DNA-coated microprojectile or *Agrobacterium*-based gene transformation delivery to generate of transgenic plants [27].

## PLANTS WITH PRECISE GENOME EDITING

With developments in the recombinant DNA technology field, it became feasible to produce precise modifications of the plant genetic material. Techniques for effective genome editing involve the Zinc Finger Nucleases

(ZFNs) [65], TALENs (Transcription Activator-Like Effector Nucleases) [66] and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) [67] are two examples of transcription activator-like effector nucleases. These strategies are generally according to a common concept, Double-Strand Breaks (DSBs) formation, supported by the addition or removal of targeted DNA sequences while DNA repair is being performed. Such strategies are thus extremely reliable and enable scientists to perform certain modifications at specific positions. Despite that, each of them has a special approach to perform DSBs. ZFNs, for instance, identify a DNA stretch of (18-36 bp), whereas TALENs involve (17-18) repeats of (34) residues, this means that TALENs are extremely precise and effective compared to ZFNs. Both strategies use the restriction enzyme Fok1 to generate DSBs at the targeted positions in the genome in order to activate the DNA repair mechanism, through either non-homologous end-joining (NHEJ) or homologous recombination (HR) [68]. Synthetic ZFNs are more expensive than TALENs and need manual manipulation to improve processing, making it time-consuming. CRISPR/Cas9 method is a prokaryotic immune system that has 40% bacterial sequence and 90% archaeal genome. It splits the target sequence using RNA to protect the organism from plasmids and viruses [69]. CRISPRs are short nucleotide sequences that range in length from 24 - 48 bases and are interspersed in the foreign DNA of former attackers to which immune responses are being performed [70]. Protospacers are such spacers, and they're closely correlated to the protospacer adjacent motif (PAM). CRISPR-related (Cas) genes encode a distinct family of endonucleases, Cas9, that utilizes two different forms of RNAs – CRISPR RNA (crRNA) and trans-activating RNA (tracrRNA) to split at an intended location in a genome that complements the crRNA. A particular sequence and a special secondary structure is required to connect Cas9 to crRNA, like hairpin. Such two elements are commonly joined together to a single synthetic user-specific RNA molecule defined as a Guide RNA (gRNA). Genomic DNA splits by Cas9 with pinpoint precision three nucleotides upstream of the PAM module on complementary strands, and 3–8 bases upstream of PAM module on non-complementary strands. [71]. The DNA repair system would then fix the breaks, either via HR or NHEJ, as with ZFN and TALEN. The CRISPR/Cas9 technique, in compared to ZFN and TALEN, provides a greater level of specificity. Another advantage of CRISPR/Cas9 over genome-editing strategies based on ZFN and TALEN is the ability to perform modifications at multiple sites through co-expressing a variety of multiple gRNA molecules [71]. Unfortunately, CRISPR/Cas9 strategy has also drawbacks like off-target effects [72]. According to research those off target effects could be significantly decreased with proper utilization of binding regions able to tolerate mismatch and the PAM module. Similarly, a greater gRNA-to-Cas9 ratio has just been demonstrated to decrease off-target effects significantly. Another study found that the expression of the mutant Cas9, Cas9D10A, decreased off-target effects by 50-1,500 times [73]. Such methods have been widely employed to develop various genetic manipulations kinds, like point mutation, genome destruction, and gene integration [74]. When using CRISPR/Cas9 to introduce transgenes at a specific place, a DNA donor repair template with an additional DNA fragment flanking the desired gene is required to allow genome homologous recombination. Despite this, homologous recombination is rare in HR-based gene introduction. For example, this method was recently utilized to introduce the PAT gene into maize at a frequency of 0.7% [75]. CRISPR from Prevotella and Francisella 1 (Cpf1), an engineered Cas9 protein that recognizes PAM on the template strand of the gRNA-binding strand, produces staggered DSBs, allowing NHEJ to insert foreign DNA with suitable sticky-ends [76]. The main transformation process depends on *Agrobacterium*, that leads to the blind incorporation of transgenes and could lead to DNA reconfiguration and other undesirable phenotypes. Furthermore, even in a stable line, epigenetic alterations of external sequences may limit transgenic expression. For instance, regardless of the fact that all plants have inherited the transgene, in the progeny of the transgenic Arabidopsis plant line, 50% of the plants did not show the desired phenotype. [77]. As a result, it is challenging to recover transgenic plant lines with the intended phenotypic and little unwanted genetic modifications. Problems besides off-target effects involve the transfer methods for gRNA expression and Cas9 cassettes and comparatively ineffective editing in certain plant species' germline cells [78]. Non-nuclear genomes, like as chloroplast genomes, were used to solve most of the problems associated with nuclear transformation. Chloroplast genome transformation is becoming a viable alternative to nuclear transformation for developing desired plant features [79].

## SELECTION MARKERS AND ELIMINATION METHODS FROM TRANSGENIC PLANTS

Transgenic plants generation greatly depends on various antibiotics which are employed as selection markers to recover the transformants. The most widely used selection markers are kanamycin (typically for nuclear transformation) and spectinomycin (usually for chloroplast transformation). They all part of the aminoglycosides class of antibiotics and are derived from two distinct Streptomyces species. Because they bind to 30S bacterial ribosomes and chloroplasts/mitochondria, they destroy untransformed cells by suppressing protein production. Hygromycin B is popular selection marker that damages both prokaryotic and eukaryotic cells by preventing tRNA from interacting with ribosomes. In nature, there are various enzymes used to manipulate such antibiotics, that constitute the foundation of the selection mechanism to recover the transgenic plant lines. For instance, neomycin phosphotransferase II (NPT-II) or aminoglycoside 3′-phosphotransferase II (APH-II) naturally produced from Streptomyces fragdiae catalyzes phosphorylation of the 3′-OH amino-sugar group of aminoglycosides, including paramomycin, neomycin, and kanamycin, making them incapable of binding to ribosomes. The NPT-II-coding gene, neo, is the earliest that is utilized as a selection marker for genetic engineering in plants. Likewise, aminoglycoside 3′-adenylyltransferase gives tolerance to streptomycin and spectinomycin through adding the ATP moiety to them. Hygromycin B phosphotransferase detoxifies hygromycin molecules by catalyzing 7′-OH group ATP-dependent phosphorylation It's a

selection marker with a wide range that is employed efficiently in a great variety of plant species. When NPT-II was never as effective, such as in monocots, the hygromycin-based selection method was especially beneficial. Selection markers that can offer antibiotic tolerance, herbicide resistance, reliance on a carbon supply, or the capacity to develop photoautotrophically serve as an important method in the synthesis of transgenic plants [80]. Because of the strict selection, antibiotic-based markers have become the most often used selection markers for recovering transgenic plant lines. Nevertheless, Overuse of antibiotics introduces safety and health issues and thus it is another limitation for commercial application areas of genetically modified plants. Aside from safety issues, the marker protein may present a metabolic burden through employing a large amount of the plant's protein synthesis capacity [81]. Nevertheless, when a stable plant line is already formed, antibiotic resistance genes do not perform any function. Consequently, in order to enhance the market adoption of genetically modified plants, marker genes are omitted from the ultimate transgenic plant line. The marker genes elimination further facilitates the regeneration of markers for repeated transformation processes [82]. There are several methods for eliminating markers from nuclear [80] and genomes of plastid [82]. The Day Laboratory developed a method for eliminating marker genes from chloroplast genomes using homologous recombination. Small direct repeats flank the marker gene in this strategy. Crossover between such repeats will ultimately remove the marker gene [83]. The gene frequency removal relies on the portion of direct repeats, and their arrangement will control the gene removal pathway. For instance, in Chlamydomonas, the transgene was effectively eliminated is (483 bp) repeat, whereas the (230 bp) repeat has been ineffective [84]. Because the approach depends on homologous recombination, it leads in a genetically unstable transplastomic line, as well as a separating ptDNA (plastid DNA) population, making it challenging to produce homoplastomic marker-free plants. The next strategy depends on phenotypic selection, which involves using a photosynthetic gene which expresses a chlorophyll-deficient phenotype with pale-green leaves. The mutants are retrieved by providing a wild-type clone of that specific gene [85]. Because that strategy involves previous isolation of the aadA knockout in the photosynthetic gene, it brings additional efforts to produce the desired plants. The CRE–loxP recombination system is used in the other method to eliminate marker genes from transgenic plants. A 34-bp sequence from the P1 bacteriophage is LoxP [86], that is identified by a 38.5-kDa CRE protein expressed from the cre gene [87]. The loxP component is made up of two 13-bp repetitions separated by an 8-bp spacer region. If the loxP locations are in the same orientation as the repeat sequence, the recombination reaction will remove any DNA between them. Recombination reactions are regulated by CRE recombinase. Such system was employed in plants to eliminate genes from the nucleus [88] and also from plastids [87] by either constant or temporary expression of the cre gene by the nucleus. Nevertheless, such strategy was troubling because of the existence of pseudo-loxP locations in the genome of plastid, that caused the depletion of ptDNA owing to increased recombination activity [87]. A further alternative strategy of the CRE/loxP method is the use of phiC31 phage site-specific integrase (Int), that facilitates recombination between bacterial (attB) and phage (attP) attachment sites. In a similar manner to the P1 CRE-loxP-mediated technique, this method of eliminating markers is effective. In this strategy, the marker gene is flanked by attB and attP locations, and if marker gene elimination is wanted, Int could be produced from the nucleus either permanently or temporarily, and would then be introduced into plastids when the elimination of marker genes from plastome is desired [89]. Another approach for recovering marker-free plants is to use super binary vectors with two separate T-DNA sections on the same expression vector for plants, one for the expression cassette and one for the selection cassette. Such two T-DNA regions would contribute to insertions at different sites in the genome. Separation of the consequent generation would lead to the segregation of marker-free transgenic plants [90]. Likewise, another version for such technique is the co-transformation by physical means of two distinct plasmids, one containing the gene of interest whereas the other containing the selection marker. Both of them would be inserted at two distinct positions in the genome and separate independently. Consequent screening of the segmenting population facilitates the detection of marker-free transgenic plant lines. Such a strategy was also utilized in the laboratory for recovering marker-free transgenic rice lines that express the Xa21gene which makes the bacteria resistant to blight [91]. While a variety of marker elimination techniques are currently applicable, the production of transgenic plants that are free of markers is a big challenge. As a result, in order to develop transgenic plants, non-antibiotic marker methods based on photosynthesis or metabolism could be used. Nevertheless, lower selection pressure opposed to the antibiotic-mediated selection method represents a greatly challenging task. It is predicted that the production of successful selection approaches dependent on native plant genes can increase the public support of genetically modified crops [79].

## IMPROVING PHOTOSYNTHESIS WITH TRANSFORMATION

Under agricultural field circumstances, photosynthetic efficiency is a major target for increasing crop yield potential. The constitutive maize *GLK* genes expression, which encode Golden2-like (GLK) transcription factors that control certain initiated processes through promoting target genes which encode chloroplast-localized and photosynthesis-associated proteins, in rice is demonstrated to result in high amounts of chlorophylls and pigment-protein antenna complexes. Consequently, the light harvesting capacity by photosystem II in field-grown plants is improved. Such improved photosynthetic capability of field-grown transgenic plants leads to elevated carbohydrate rates and also raises the rates of vegetative productivity and cereal yields 30–40% [92]. Furthermore, the pyruvate-phosphate dikinase (C4-PPDK) gene was successfully transferred to a $C_3$ model plant in multiple abiotic stress settings in an ambient and enhanced $CO_2$ environment from Suaeda monoica, which includes a single-cell $C_4$ photosynthetic

pathway without Kranz architecture. Over-expression of *SmPPDK* has regulated the $C_3$ transgenic plant growth, promoting their photosynthesis via decreasing the photorespiration under stress environments [93]. In *Nicotiana benthamiana* plant, down-regulation of fatty acid desaturase gene (FAD7) revealed greater photosynthetic level and PS-II effectiveness under increased temperature conditions as opposed to control transformed plants because of the greater ratio of dienoic to trienoic fatty acids improving membrane stability. Therefore, it was concluded that the modification in the structure of fatty acid in thylakoid membrane could decrease the oxidative stress, increase the photosynthetic level and PS-II effectiveness in plants under elevated temperature conditions [94]. Over-expression of plant ferredoxin-like protein (PFLP) gene, cloned from sweet peppers, in rice plants resulted in improved photosynthesis efficacy compared to the wild type plants. Furthermore, a significant increase in the photosynthetic products, including glucose, fructose, sucrose and starch contents in *pflp* transgenic lines have been observed. Hence, it has been inferred that the constitutive expression of *pflp* could increase rice plant biomass via improving the efficiency of photosynthetic carbon assimilation [95]. Choline oxidase gene *(codA*) has been cloned from *Arthrobacter globiformis*, that has been manipulated to target both chloroplasts and cytosol, and then transferred to tomato (*Solanum lycopersicum*). Transformed plants have shown increased amount of glycine betaine, whereas wild type plants have no detectable glycine betaine. In addition, opposed to wild type plants, the photosynthetic rates and antioxidant enzyme activities have significantly been increased, whereas the reactive oxygen species have been decreased in the transgenic plant leaves under the salt stress conditions [96]. Moreover, it has been proposed that the over-expression of SikRbcs2, a small subunit of Rubisco, could make the photosynthesis process in the transgenic tomato unaffected by low temperature. Also, it could achieve a stable photosynthesis, improve scavenging ROS capability of transgenic tomato, maintain membrane stability, and make the transgenic plant cold tolerant [97]. Sedoheptulose-1,7-biphosphatase (SBPase) gene has been cloned from *Brachypodium distachyon* and transferred to wheat. Transgenic plants with raised SBPase protein rates have shown improved leaf photosynthesis and enhanced total productivity and dry seed yield [98]. Likewise, expression of *Populus* SBPase gene in *Arabidopsis thaliana* has resulted in increased leaf area, root length, and plant height. *SBPase* has been indicated to perform a significant function in producing ribulose triphosphate, starch and other polysaccharides, and also it has resulted in accumulated amount of carbohydrates and thus regulated photosynthetic capability [99]. In recent years, Gene for cucumber transglutaminase (CsTGase), regulator of post-translational modifications of protein which protects the plant from various environmental stresses, has been over-expressed in tobacco plants leading to improved salt-induced photo-inhibition, raised levels of chloroplast polyamines and increased D1 and D2 proteins abundance. TGase has also activated the expression of photosynthesis associated genes and remodeling of thylakoids under the optimal environments [100]. In order to establish homozygous over-expression lines, the gene for ZmNF-YB16, a basic NF-YB super-family member and a member of a transcription factor complex consisting of NF-YA, NF-YB, and NF-YC in maize, was transferred to the inbred maize line B104. The results have indicated that *ZmNF-YB16* could regulate the expression of certain genes essential for photosynthesis, the cellular antioxidant capability, and the endoplasmic reticulum stress response; consequently, it can make the transgenic plants resistant to drought [101]. In transgenic tobacco plants, the over-expression of maize *psbA*, which expresses D1 protein, has displayed raised D1 protein levels and improved drought stress tolerance. As a result, it can be concluded that *psbA* has decreased the photosynthesis reduction and thus caused the transgenic tobacco plants tolerant to drought stress conditions [102]. *Nicotiana tabacum* solanesyl diphosphate synthase 1 (NtSPS1), the key enzyme in solanesol biosynthesis, which has also been over-expressed in tobacco, has resulted in significant enhanced leaf growth, photosynthesis, and chlorophyll content compared to the wild type tobacco lines [103]. Furthermore, B-box gene *(BBX29)* has been cloned from *Arabidopsis thaliana* and over-expressed in sugarcane (*Saccharum* spp.). It has indicated to improve the drought tolerance and delay senescence in the efficiently transformed plants under water deficit environments due to the great maintenance of water content and protection of photosynthesis system [104]. The over-expression of the Rieske FeS protein, a component of the cytochrome $b_6f$ (cyt $b_6f$) complex, in *Arabidopsis* plants has led to important effects on the quantum effectiveness of PSI and PSII, electron transport, productivity, and seed yields. Therefore, it has been indicated that the manipulation of electron transport processes can raise the crop productivity [105]. This protein has also shown to enhance C4 photosynthesis when was over-expressed in *Setaria viridis* [106].

## CHALLENGES AND LIMITATIONS

There are many aspects which restrict our abilities to enhance photosynthetic effectiveness by genetic manipulation. First, as a key metabolic mechanism which has developed over many centuries, photosynthesis is a complicated system which is profoundly incorporated into the entire metabolism of photosynthetic organisms. Photorespiration substances possess an effect on the capability of an obviously unassociated mechanisms, like nitrate assimilation [107]. Altering one component affects other reactions. Information on the integration of metabolism and regulatory pathways remains poor and demands more research [108]. The second restriction is attributed to the applicable methods. Since photosynthetic features are always expressed in both nuclear and plastid genomes, advanced techniques to transform both genomes are required. In addition, such approaches involve the insertion of long DNA fragments which encode many proteins. Nuclear transformation methods encounter a variety of challenges. Genes inserted are vulnerable to silencing, and sufficient transgene expression is almost always not achieved. Information about promoters, terminators, transport signals, centromere and telomere sequences is still limited. Plastid transformation methods allow the integration of several transgenes with higher accuracy and also the

achievement of great expression rates; however, transformation procedures were only designed for a limited number of organisms. Plastid transformation of grains has still not been effective [109]. Even when genes have been effectively inserted and expressed, their outputs ought to be correctly folded, transferred and, in certain circumstances, constructed into functioning complexes [108]. However, advanced methods which allow accurate genetic modification were established, including the CRISPR/Cas9 method [110] and a method involving transcription activator-like effector nucleases (TALEN) [111]. The integration of plant mini-chromosomes has brought up modern opportunities for the insertion of a greater variety of genes into plant species [112].

## CONCLUSION

With raising food and fuel demands, the requirement to improve variety of essential crops with higher incomes is at the top of the priority list of agricultural research. Recently, the use of genetic engineering to enhance the level of photosynthesis as a way to increase the productivity has also come to the forefront. Latest new technologies for transformation of the nucleus or chloroplast genome have improved, making it simpler and more accurate to manipulate the photosynthesis system. These plants are supposed to show more effective photosynthesis under controlled settings, the plant farm where plants are grown in an optimal growth condition will have possible benefits of increased yields. In the coming years, the combination of a variety of techniques will significantly contribute to increase the photosynthetic capabilities and consequently crop production.

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