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## Recent Advances in Plant Biotechnology and Genetic Engineering: Applications in Agriculture

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### Abstract

Increasing population is facing the challenge for food security. Researchers are searching the convenient and fast ways for improving crop production. Basic research provides us with genetic mechanism of plants. Tools have been discovered to manipulate and alter these mechanisms to get the new and desirable products and to incorporate innovative characteristics in plants. Synthetically made promoters, enhancers and repressors for native or transgene expression regulation are some of these tools. Some tools like, transformation of plant with artificially synthesized chromosomes and linked multiple genes are gaining importance. The most advanced one is CRISPR-Cas genome editing system. To assess their implicational potential in addressing agro-environmental problems, such genomic tools should be integrated.

**Keywords:** CRISPR, gene editing, promoter, regulation, vector.

## INTRODUCTION

There are many challenges faced by the growing population. In regards of this spectrum, there is a need for food security, for this purpose, improvement in plant performance with respect to resistance against environmental stresses and improved crop production is first task (Collins, 2008). This is accomplished by the altering growth and developmental processes and metabolism of plant for improving existing gene functions or making new products. To acquire improvement in agriculture and plant science different techniques like proteomics, transcriptomics, metabolomics is required (Zaynab *et al.*, 2017). Concept of genetic engineering of plants is older than three decades (Wollenweber *et al.*, 2005). Major tools for incorporating genetic material with the help of *Agrobacterium* transformation are considered the part of genetic engineering and these techniques were developed in 1980s (Zaynab *et al.*, 2017).

All commercial transgenic crops became possible due to genetic engineering as the genome of organism can be manipulated by incorporating one or more novel genes and regulatory sequences or by down regulating the endogenous genes (Naqvi *et al.*, 2010). These tools are beneficial for insertion of few bases or a complete gene into plants but not favorable for introducing big changes like introduction of complete metabolic pathway. To control expression of foreign gene synthetic promoters are getting attention. Although there is possibility of using these genetic tools for basic plant research but this review concerned with advances and implications of these tools for agriculture and plant biotechnology.

## Gene Expression Regulation

The major challenge in plant biotechnology is regulating expression of genes so different techniques can be used such as RNA sequencing (Zaynab *et al.*, 2018). Scientists have developed the concept of synthetic promoters and synthetic repressor and enhancer (regulatory elements) for native and transgenes expression regulation.

## Synthetic Promoter

Mostly promoters that are part of the cellular genome are weaker than the vector-based promoter but longer than ~ 1 kb. While the promoters used as biotechnological tools are designed stronger and shorter for specific developmental or tissue specific stage gene expression (Venter *et al.*, 2007). These promoters are designed with help of computers and act as regulatory elements, as a binding site for regulatory proteins. These are synthesized by keeping in mind the DNA sequence that found in native promoters, but condensed and rearranged in such a way that it become a new sequence that doesn't exist natively. Early studies has demonstrated the advantages and

potential of synthetic promoters in commercial applications e.g. pathogenic bacteria were detected by plants using multimerized regulatory cis-elements and signaling pathway (Liu *et al.*, 2013). In transgenic tobacco and *Arabidopsis thaliana*, synthetic promoters were induced by plant pathogenic bacteria, which enable the plant to produce defense compound signal that are the main regulatory elements in promoter induction, these promoters derive the expression of a reporter gene that encode fluorescent protein. Availability of modeling computer softwares and regulatory cis-elements has limited the engineering of synthetic promoters. Deconstructive analysis was used to manipulate the function of promoter by 5' deletions in history. While modern approach is constitutive analysis in which regulatory motif identified through database motif analysis is added (Mehrotra and Mehrotra., 2010). Apart from this, these promoters can be developed by inserting the functional part or motif to the native promoter without using the computational databases e.g. *Mac16* and *Pcec15* are two promoters synthesized by just introducing the enhancer domain upstream side of the native promoter. These synthetic promoters are responsible for the great expression of reporter genes in many plants. Using integrative approaches can enhance the knowledge of promoter synthesis that rarely found in nature and interaction between promoter and regulatory elements.

## Synthetic Enhancer and Repressor

In plant genome, these synthetic enhancer and repressor are used to regulate the expression of native or transgenes (Petolino and Davies., 2013). For targeted gene expression, many fusion proteins with binding and catalytic domains are produced which induce targeted gene expression under control of synthetic promoters. In tomato *Bs 4* gene and *EGL3* and *KNAT1* gene *Arabidopsis* were induced by TALEs that having their activating domain. These factors worked under control of *CaMV 35S* promoter. Expression of the genes resulted in alternation in *Arabidopsis* leaf morphology and trichome no. in tomatoes. TALE-TFs and ZF-TFs are important tools for transgenes endogenous genes expression regulation and can be manipulated at crop improvement level. These factors can act as master switches for development, growth and metabolic pathways in plants (Gupta *et al.*, 2012). While there are certain factors which should be considered for designing of these factors i.e. targeted promoter binding site location, uniqueness and sequence, off targeted effects and activation domain choice.

## Transformation Methods and Vectors

Incorporation of multiple genes or complete metabolic pathways is needed for broad level implications of genetic engineering. To achieve this goal our potential is totally

dependent on traditional methods which includes several rounds of transformation, conventional breeding and transgenes locating on multiple loci. Different methods of transformation are used like nuclear genome transformation, virus-mediated transfer, TAC vector, BIBAC vectors, MISSA-assisted transfer and Plant artificial chromosomes. Among all Plant artificial chromosome has gained much importance as alternative transformation and expression vector having greater role in next generation based transgenic technologies.

### Plant Artificial Chromosomes

For alternative transformation and expression vector artificial chromosome can play vital role in upcoming transgenic technology. These artificial chromosomes can help to overcome the problems relating to linkage of undesirable loci, disruption of endogenous genes and position effects. In plant biology new stack of genes, new traits and new metabolic pathway can be incorporated by using artificial chromosome and hence breeds of crops with new traits can be achieved. Three elements i.e.: centromere, replication origin and telomeres are required for fully functional minichromosome (Carlson *et al.*, 2007). The most interested and poorly studied part is centromere which is majorly composed of repeat elements. Two approaches namely top down and bottom up approaches, have been developed to create minichromosome and bottom up approach, very well promising approach, being used for assembly of centromere and telomere sequences containing genomic DNA with origin of replication and selective marker gene. By bottom up approach, chromosome based vector for genetic transformation is possible by modifying already existing chromosome. Truncation of maize chromosome occurred, for example, when telomeric repeat of 2.6 kb length from *Arabidopsis thaliana* was inserted (Yu *et al.*, 2007).

The transgenic telomeric sequences initiate healing by recruiting telomerase and their binding proteins. Additionally telomere sequences could be flanked by points of site-specific recombination similar to those mediated through FLP-FRT system of Cre-LoxP system. This chromosomal truncation can be resulted in loss of gene as well as genome instability too (Houben *et al.*, 2008). So in this case another chromosome, minichromosome, is ideal target of telomeric truncation due to their dispensable nature. Successful transmission of engineered miniB chromosomes have been achieved with 12-39% transmission rate in maize and this percentage is required to increase for useful field application of this approach. Aside from the fact that mini chromosomes are being promising but they not yet have a reasonable effect for agricultural uses.

### Transgene Bio-confinement and Removal

Transgene escape through pollen or seed, a major environmental and regulatory concern in transgenic crop cultivation. As selectable marker genes are not required after transformation so they can be removed from transformed final product (Daniell., 2002). Transgenic bioconfinement approaches, including transgenic mitigation, apomixes, female or male sterility and cleistogamy are suggested to minimize transgene escape. Transgene or selectable marker-gene removal could be achieved via site-specific meganucleases or ZFNs (Daniell., 2002).

Additionally recombinases also used to denature DNA on genetically engineered recognition sites. There are two types of recombinases, Tyrosine recombinases use catalytic tyrosine residue for cleavage activity, such as Cre, FLP, with identical DNA recognition sites of loxP, FRT, RS respectively (Yang *et al.*, 2013). Serine recombinases: which confer irreversible excision in absence of exonuclease, a helper protein. These are CinH, ParA, Bxb1, PhiC31 with recognition sites of RS2, MRS, "attB and attP" (differ in sequence) "attB and attP" respectively (Lloyd *et al.*, 2001). In a construct having transgene flanking with specific recognition sites, recombinases can be expressed under control of tissue specific or inducible promoter (Jamal Khan *et al.*, 2011). If removal of transgenic traits to get seeds and pollens free of transgenes is achieved with approximately 100% efficacy, then transgenic bioconfinement should be valuable helper for crop improvement.

For plant genome alterations, genome editing (GE) consists of several techniques of great value. These techniques being useful for regulating genetic expression at specific site and enable us to develop new insights into the plant functional genomics. Random mutagenesis or low-efficiency gene targeting has been effectively used in plant cell lines or models for genome engineering (Wolt *et al.*, 2016). Endogenously targeted genome modifications can be achieved successfully by using engineered nucleases (GEEN) and programmable sequence-specific DNA nuclease etc. CRISPR (Clustered regularly interspaced short palindromic repeats) is a flexible genome editing technique to alter DNA at particular site. For gene functional analysis, CRISPR has emerged as pivotal technique by creating genetic variations at target site. By using CRISPR variations has been introduced in problematic species that previously resist genome modifications by other techniques (Bolotin *et al.*, 2005). Until now, majority of studies have been conducted on animal system. Recently CRISPR/Cas9 has been successfully used for creating genomic variations in *Arabidopsis*, sorghum, tobacco, rice thus ascertaining its applicability in both monocot and dicot plants (Qi *et al.*, 2013).

## CONCLUSION

Synthetic biology is striving with genetic improvement by reconfiguring and replacing the native genomic components using advanced tools. Old tools include synthetic promoters while, TALENS are new one gaining much importance day by day. Genomic pathways and circuits have been designed and will be applied to the plants in near future.

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## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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