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Plant Genetic Engineering, In a Nutshell

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Abstract

Plant genetic engineering involves the processes of modification of plants using genetic mechanisms in order to produce varieties of plants that have a commercial advantage over the traditional crops. This is often utilized in bringing about solutions to problems in the quality of the food matter, strongly related to increasing the photosynthetic potential and better responsiveness to pests and other physical, chemical or biological stresses that the plant communities face. There are a range of such techniques that are employed to incorporate beneficial genes to increase the crop quality and quantity. Moreover, these methods are also utilized to remove unfavourable genes that can otherwise bring about harmful effects to the plants' commercial value. In spite of the many benefits of utilizing gene manipulation in maximizing the potential of plant based economies, the realization of the drawbacks in new technology are also inevitable. Appropriate risk assessments done for regulation depend on the base of updated scientific knowledge for generation of list of possible harms to be assessed. Therefore a better understanding of the risks and benefits of using plant genetic engineering is essential in the future prospects as a successful strategy to meet the needs of the present and future generations.

Key words : *Plant genetic engineering, nucleases, transcription, virus, zinc.*

Introduction

One of the world's oldest and largest industries is Agriculture. Throughout time, in cultivating plants many techniques have been employed in order to get a larger yield of the grown crop. In this evolutionary period efforts have been taken to increasing the quality and productivity of crop.The successes of modern agriculture is accounted to the increased reliance on advanced technology, and how new technology has been integrated with the results of intensive plant breeding programs. On the other hand however these measures taken for crop improvement have also brought about many unforeseen problems which are increasing with time. In order to overcome these problems, there is a promising future for accepting plant genetic engineering through various forms of recombinant DNA technology. Many techniques have been employed in successfully implementing genetic engineering in plants, which have a wide array of applications. Despite the wide acceptance and potential success, there are however many risks that can be associated (Mantell *et al.,* 1985; Hahne *et al.,* 2019).

This literature survey aims to look into the applications of plant genetic engineering, the techniques utilized and several risks associated.

Applications of plant genetic engineering

Although the major use of plants is as a food source, there are many other uses of the harvest, including ornamental and biofuel purposes. Accordingly, improvement of plants helps to thrive in all these sectors. Plant genetic engineering involves the insertion of alteration of the genetic structure of plants in order to increase their productivity, by the transfer of favourable genes to otherwise normal genetic structure or deficiencies as below (Low *et al.,* 2018).

The improvement of plants can be done in terms of different biological, physical or behavioural factors of plants. Photosynthesis is regarded the most important function that is providing the most contribution to humans, animals and the entire environment. While some plants possess the ability to photosynthesize in larger capacity, there are some plants that are not able to. These differences lie in the ability to carry out different steps of photosynthesis, most remarkably in the electron transport chain. The improvement of photosynthetic capabilities can be attributed to the exchange of photosystem components between different plants. This would thereby result in the optimization of electron transfer (Zu *et al.,* 2010). Nitrogen Fixation is an important phenomenon, which is coupled with the bacteria association. This cannot be achieved if the specific bacteria are not present. The new technologies have looked at inserting the genes of bacteria responsible for nitrogen fixation to plant genes. This gives the way for plants to carry out nitrogen fixation without the need of these bacteria.The seeds of legumes and cereal grains provide with about 70% of the dietary protein requirement. When the seed develops, storage proteins are synthesized and

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accumulated in the seed, which allows providing the proteins required during early seed germination. This gives rise to the availability of high levels of such protein as an enriched amino acid source for both human and animal consumption. However, if there are deficiencies of seeds in certain essential amino acids this results in cereal grains or legumes limiting amino acids from other sources (Jacoby *et al.,* 2017).

Gene modification can be done in order to increase the storage of these proteins and overcome deficiencies. Another application of plant genetic engineering lays the improvement of pest and pathogen resistance. With increasing use of pesticides, most plants have reduced immunity, as the pathogens develop resistance against these chemicals. Therefore, the transfer options now look at injecting plants that have reduced resistance with refined gene fragments from those that are highly resistance to such pathogens. This phenomenon of inducing resistance in plants holds a resemblance to vaccination. Stress tolerance is another significant contributor to the success of plants. Plants can often be faced with many stresses. These stresses can range from chemical, physical, to physiological aspects. The success of thriving of the plant lies in how well the plant responds to these burdens or its tolerance range. While some plants intrinsically have high levels of stress tolerance, there are many plants that do not have this ability. The modern technology aims to induce these stress tolerance genes in those that do not possess them, thereby giving rise to plant communities that can withstand many changes in the environment ensuring their survival (Barton and Brill, 1983; Kirschbaum, 1985; Iqbal *et al.,* 2021).

Another use of plants in addition to the traditional agriculture is the production of ethanol from plant biomass. This is a sector with increased importance and much advancement in plant genetic engineering is developing to reduce the costs associated with this biomass conversion. The applications have a range of investigations. These includegeneration of plants that can produce enzymes such as cellulase and ligninase or those that produce reduced lignin content. Plant genetic engineering therefore also serves for the betterment ofthe biofuel industry (Sticklen, M., 2006)

Techniques

The genetic modification of plants is addressed via a range of genetic engineering technologies. These often include techniques such as CRISPR/CAS9, CRISPR base editors, zinc finger nucleases, transcription activator-like effector nucleases, RNA interference and virus-induced gene silencing amongst many others. These techniques play a significant role in bringing about the success of plant genetic engineering to meet the rising needs crop optimization (Dönmez *et al.,* 2016)

CRISPR/CAS9

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR Associated Protein9 often referred to CRISPR/Cas9 can carry out the rapid editing of genomic DNA with high degree of efficiency. Utilizing the repairing of DNA double strand breaks induced by CAS9 and single-guide RNA (sgRNA), CRISPR/Cas9 allows precise mutation of one specific gene, which has been successfully applied in plant genetic modification.Upon selection of the target sequence, the gRNA is designed and if needed the optimization for gRNA promoter and Cas9 gene sequence for the target plant is carried out. Then the CRISPR/Cas9 vector is constructed and plasmid is transformed. Following the culture of T_0 generation the seedlings are screened and the transgenic plant is developed. This technique offers a number of advantages as it requiring only a crRNA for the targeting and causes a stunned cut end. This also increases the chance of having repeated rounds of DNA cleavage as it cleaving the DNA at 18-23 base pair downstream from the site of point of mutation. Further, alternate target sites in T rich regions are also provided (Butt *et al.,* 2020)

CRISPR Base Editors

An extended advanced version of CRISPR/CAS9 systems is one that introduces a change of single nucleotide at the target loci. This is termed as CRISPR Base Editors. In addition to the initial steps of CRISPR/CAS9 such as the selection of the target sequence and design of gRNA, the CRISPR Base Editors involves the selection of Cas9 Variant and Deaminase, and its vector construction. The transgenic plant is then created by undergoing the steps similar to the CRISPR/CAS which include the plasmid is transformation, culture of T_0 generation and the screening of seedlings. This CRISPR Base Editors method allows for the simple construction with the precise base editing without the need of double stranded break formation or a donor DNA template as opposed to the CRISPR/CAS method. This technique is successfully applied in plants like wheat, rice, maize, and tomato with high degree of success. However, it is noted that many advanced systems should be acquired for more effective and efficient editing of Cas9 variants such as SpCas9, SaCas9, SpVQR-Cas9, with different PAM (protospacer-adjacent motif) specificities to obtain successful results (Gürel *et al.,* 2020).

Zinc Finger Nucleases (ZENs)

The Zinc Finger Nucleases (ZFNs) are artificially generated restriction enzymes that have been engineered

for the genome editing field. This method includes a zinc finger DNA-binding domain and a DNA cleavage domain. This domain is usually *FokI*. The DNA-binding domain comprises of three to four zinc fingers proteins with conjugated Cys2His2 motifs. Each of these recognizes a specific nucleotide triplet, which is based on the residues found on their alpha-helix. The DNA cleavage domain arises from the *FokI* restriction endonuclease. This endonuclease should dimerizein order to cut the DNA in a nonsequence-specific manner which induces DNA double strand breaks (DSBs). These DNA double strand breaks usually repaired by two pathways available. These repair pathways are thenon-homologous end joining (NHEJ) and homology directed repair (HDR) pathways. The non-homologous end joining pathway leads to insertions, deletions, or substitutions of nucleotides in the regions that are broken. On the other hand, the homology directed repair pathway employs homologous DNA as a template in order to restore these DNA double stranded breaks. This allows for the repair in precise and controllable means.This Zinc Finger Nucleases technique is therefore considered as a promising tool for editing the plant genome in order to improve the quality of crops (Miller *et al.,* 2007).

Transcription Activator-like Effector Nucleases

Transcription Activator-like Effector Nucleases (TALENs) is another highly efficient and versatile tool that is used successfully in the field of genome editing. After selection of target sequence, the Transcription Activator-like Effectors are designed, followed by the construction of the nuclease vector. These TALENs usually consist of TALE DNA-binding domains and restriction endonuclease *FokI* cleavage domains. These are responsible for introducing of DNA double stranded breaks, followed by the endogenous repair of these breaks. This technique of using TALENs is used broadly in the plant gene function research in addition to plant breeding methods (Wei *et al.,* 2013).

RNA Interference

The RNA Interference (RNAi) is a classical technique that is used to generating sequence-specific gene knock-down or knock-out consequences. This is carried out by introducing specific double-stranded RNA (dsRNA) into the target cells in plants. This technique gives rise to impairment of the endogenous gene functions of cells in a quick and precise manner. Also this RNAi technique is regarded as an affordable method. This technique has led to the discovery of function-enhanced novel crops including nicotine-free tobacco, non- allergenic peanuts, and decaffeinated coffee. Due to its high efficiency, quickness, and general affordability, RNAi has become a promising approach for high-tech crop improvement (Mansoor *et al.,* 2006).

Virus-Induced Gene Silencing

Virus-induced Gene Silencing (VIGS) is also another rising technique in plant genetic engineering that serves to the removal of genes that otherwise can bring about harmful effects. This technique employs small interfering RNAs (siRNAs) that are used to accomplish degradation of mRNA, suppressionof translation or the inhibition of targeted genes of transcription. In this system viral vectors are used to carrying a target gene fragment to produce dsRNA which can give rise to RNA-mediated gene silencing. In this method, after viral vectors are transferred into host cells, Small interfering RNAs (siRNAs) are produced by using RNA-dependent RNA polymerase (RDRP). This follows their incorporation into the RNA-induced silencing complex (RISC). The degradation and/or suppression of target mRNA is guided by the antisense strand of the siRNA. This can also be achieved by the inhibiting of transcription of the target mRNA by RNA-directed DNA methylation (RdDM) in the promoter region.Virus-induced Gene Silencing show many benefits in comparison to other techniques used in plant genetic engineering. Since the target gene fragment can be assembled by the direct cloning within the virus vector it is a safe and easy method, in contrast to using inverted repeats which are unstable during multiplication in bacterial hosts. This technique is also quick as the VGIS vectors can be introduced by mechanical inoculation or biolistic bombardment. Further, implementing VGIS method is highly cost-effective. This method is therefore used as a successful alternative to the RNA interference method explained in previous section (Velásquez *et al.,* 2009; Dey *et al.,* 2017).

Challenges

Despite the discussed advantages in available techniques of plant genetic engineering, studies have identified a number of ways in which genetically engineered plants could potentially bring about adverse impacts. These arising problems have impacts on human health and the environment.Most of the potential dangers to human health lay in the growth and consumption of the genetically engineered plants. The risks posed in respect of these plants depend widely on the new genes that are introduced into them (Tsatsakis *et al.,* 2017).

A striking burden posed by transgenic plants is the introduction of new allergens into foods. These can bring about allergic responses to foods that would generally be non-allergic. This can be seen in a situation where the gene responsible for production of allergenic proteins of milk is transferred to vegetables which are not otherwise

allergenic. Development of antibiotic resistance, production of new toxins and concentration of toxic metals are also prominent unforeseen risks that can be associated with the production of genetically engineered plants. Furthermore, there is a risk of unforeseen genetic mutations that can lead to the creation of new viral strains that we do not have any information on (Maghari and Ardekani, 2011; Bauer-Panskus *et al.,* 2020). Therefore it is important to re-think and visalise the probable ill-effects that can arise from plant genetic engineering.

Conclusion

Manipulation of genes is seen as a promising area in the production and propagation of plants for various financial gains. The multitude of methods available to carry out genetic engineering of plants, are in fact diverse. Each of these techniques has unique properties and their advantages and disadvantages over other available techniques. Research is still underway to assess the possible risks and harms associated with the use of these techniques. However, in order to move forward constantly it is important to overcome these risks and outweigh the recognized disadvantages. Thereby, the future is made clear for the safe application of genetic engineering in plants.

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