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AGRICULTURE BIOTECHNOLOGY USING MODERN GENOME EDITING TECHNIQUES

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ABSTRACT
Agriculture biotechnology is also known as green biotechnology is the use of new scientific techniques based on our understanding of DNA to improve and modify living organisms, plants, animals and microorganisms. This can be achieved by using scientific tools, techniques including genetic engineering, molecular marker, molecular diagnostics, vaccines and tissue culture. Genetic engineering for crop improvements, agronomic and nutritional traits has been widely used. This involve the introduction of novel trait into the crop through the manipulation of its genetic material and form the transgenic or Genetically Modified (GM) crops. In this mini review, we have put light on the process and application of genome editing method of crop biotechnology.

Keywords: Crop biotechnology, Genetically Modified (GM) crops, genome editing, Crop modification techniques.

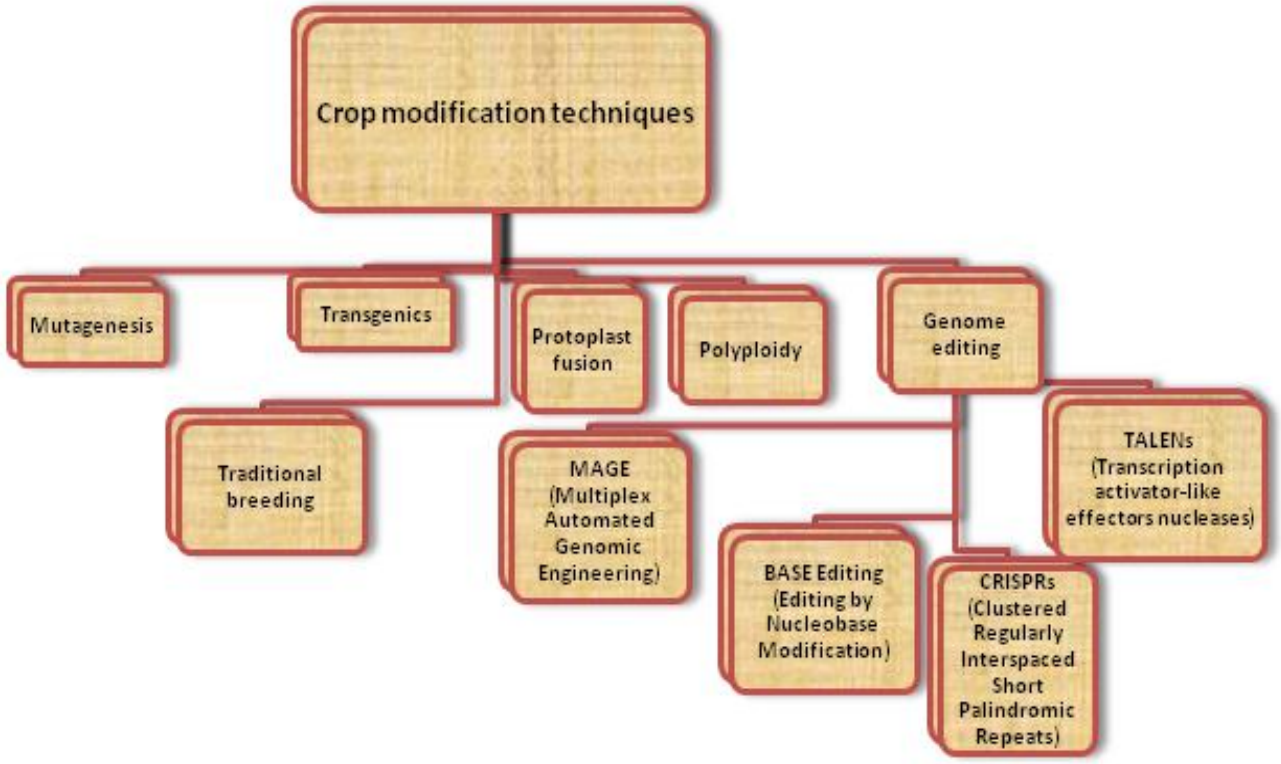
INTRODUCTION

Crop biotechnology in form of plant molecular biology came into existence in the early 1980s and has been showing tremendous growth since then.[1] The study of plant genes and genomes, joined with the improvement of techniques for the integration of modified genes into plants, ultimately led to the commercialisation of genetically modified (GM) crops in the 1990s. Whereas, in traditional breeding method two sexually compatible species crossed with each other to create a new and special variety of plants with a desired trait of the parents. For example honey crisp apple exhibit a specific texture and flavour due to the cross breeding of its parents.[2] Mutagenesis breeding involves the introduction of random mutations to plant cutting using chemicals or irradiation. Explants which express new traits are then propagated from this mutagenesis. Ruby red grape fruit and single malt scotch are both derived from mutagenesis. Polyploidy which can be induced by modify the number of chromosomes in a crop in order to influence it's fertility and size. Seedless watermelon is an example of polyploidy.[1,2] Protoplast fusion is also an effective method, in which the joining of cells or cells components to transfer traits between species. Traits of male sterility are transferred from reddish to red cabbage by Protoplast fusion. Fifth technology that falls under the umbrella of genetic engineering is RNA interference or RNAi technology in which plant is designed to produce an anti-sense RNA to a particular gene, whose expressions is then blocked via gene silencing. Another is transgenesis in which genetic material can be incorporated into the plant genome either via Agrobacterium mediated transformation or by Biolistic (green gun) to create a new variety with desired traits. For example Rainbow papaya, which is modified with a gene that gives it's resistance to the papaya ring spot virus.[3]

Crop Biotechnology - Genome Editing

The genome editing for crop improvement is experiencing rapid growth as new methods and technologies. Using genome editing to increase agriculture output is needed as the world population is expected to grow to 9.6 billion by 2050 while the amount of arable land decreases. Besides potential for boosting harvest yields, genome editing is now one of the best tools for carrying out reverse genetics and is rising as an especially resourceful tool for studying basic biology.[2]

Genome edited plants are distinguished from conventional transgenic plants as they may not integrate foreign DNA. Although genome editing can be used to commence foreign DNA into the genome, it may simply involve changes of a few base pairs in the plant's own DNA. This distinction makes genome editing a novel and powerful breeding tool that has promising applications in agriculture, especially when genome shortened crops are not regulated as genetically modified (GM) plants.[4]



Double strand break repair

Genome editing promises giant leaps familiar in advancing biotechnology, farming, and basic investigate. The procedure relies on the use of progression specific nucleases (SSNs) to make DNA double stranded breaks at target genomic loci, which are further need to be repaired by DNA repair pathways. There are two main DNA repair pathways: non-homologous end joining (NHEJ) pathway and homology directed repair (HDR) pathway. NHEJ can result in frame shift mutations that often create genetic knockouts.[3]

Engineered nucleases

In recent years, transcription activator-like effectors nucleases and clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR associated protein 9 or CRISPR from Prevotella and Francisella-1 have emerged as the preferred SSNs for research purposes.[5]

Meganucleas

Meganucleases, or homing endonucleases, are site specific endonucleases found in eukaryotes, archaea, and bacteria which recognize DNA sequences over 12 bp long. Several hundred meganucleases have been discovered and they can be divided into four families: these are LAGLIDADG, GIY-YIG, His-Cys box, and the HNH family.[5] The LAGLIDADG family consist of meganucleases I-CreI and I-SceI. Meganucleases were only use to cut single stranded DNA sequence and thus were unable of targeting endogenous genes. For example, targeted mutagenesis was successfully achieved in maize with de novo-engineered meganucleases However, DNA binding properties of meganucleases cannot be completely separated from their nuclease activity, making them difficult to engineer and use in research.

Zinc finger nucleases

Zinc finger nucleases (ZFNs) are special kind of enzymes used in genome editing. They function as dimers and each monomer is form by fusion of a zinc finger DNA binding protein domain and a non-specific FokI nuclease protein domain. Some researches revealed that the zinc fingers recognizes a long stretch of DNA by putting multiple zinc fingers together for editing process.[3] However, ZFNs based on modular assembly typically have poor activity and high toxicity suggesting there is context dependency among neighboring fingers.[2,4,5]

TALEN

The engineering transcription activator-like (TAL) effectors ate very effective to search specific DNA sequence in host cell and hence good for DNA targeting. TAL effectors in can be introduced into plant host cells by the vector bacterium *Xanthomonas* via the type III secretion system, where they alter host gene expression and promot the formation of proteins as per need of parasitic bacteria. Once entered in the nucleases, the TAL effectors immediately bind with target gene's promoters 'and activate transcription.[6] Using a β-glucuronidase (GUS) reporter in tobacco, some researchers discovered repeat variable diresidue (RVD) at positions 12 and 13 of each repeat determines nucleotide binding specificity. The creation of a new

kind of SSN called TAL effector nuclease (TALEN), is based on the union of a Fok1 nuclease enzyme domain to the DNA binding TALE repeats on the host genome.[7] TALENs have many advantages over ZFNs. For example, TALEs are less toxic than ZFNs and secondly, these are comparatively easy to engineer. Although, the repetitive sequence of TALE makes them hard to create via automated polymerase chain reaction (PCR).[8] This was addressed with the development of multiple assembly methods mostly based on Golden gate cloning, which furthered rapid adoption of TALEN technology for genome editing in many organisms including plants.[6,9]

CRISPR

Another genome editing tool introduced was "Clustered regularly interspaced palindromic repeats (CRISPR)" this had been found to function as an adaptive immune system in bacteria and archaea against invading viruses, phages and plasmid.[2] This series of CRISPR associated (Cas) proteins cleave viral DNA and help to protect bacteria and then use certain Cas9 protein(s) paired with RNA transcribed from the viral DNA library. Class 2 CRISPR-Cas systems utilize single-protein effectors, such as Cas9, for DNA targeting. Cas9 is composed of two endonuclease domains, HNH and a RuvC-like domain that each cut one strand of DNA.[3] It has been showen that Cas9 element of *Streptococcus pyogenes* could be paired with a synthetic single guide RNA (gRNA) to create a targeted DNA DSB in vitro in vital medium like *Escherichia coli*. Shortly after, CRISPR-Cas9 was demonstrated as a powerful RNA-guided SSN for genome editing in human cells. Although off target effects have been a concern, the simple design and ease of vector construction has dramatically increased the number of genome editing studies using CRISPR-Cas9 in plants.[2-4]

Multiplex Automated Genomic Engineering

One distinct advantage of CRISPR-Cas9 over TALEN is the ability to multiplex automated genomic engineering. By expressing multiple gRNAs that independently pair with Cas9, multiple target sites can be mutated in a single cell.[9] This multiplexing property of CRISPR-Cas9 has enabled targeted deletion of large chromosomal segments containing multiple genes in rice and in Arabidopsis. Concurrent targeting of a number of genes can result in more than one improved features in crops, especially those which are of high demand in market and can also be used in basic research to deduce the role of each gene in a complex network.[8,9]

Precision and efficiency of engineering nucleases

Meganucleases method of genetic engineering is the least efficient of the methods mentioned above. Because of the nature of its DNA-binding elements, it is limited to recognize one potential target every 1000 nucleotides. ZFN was developed to overcome the limitations of meganucleas.[10]

TALE nucleases being the most accurate and unambiguous method yields a higher efficiency than the previous two methods. It achieves such competence because the DNA binding component consist of an array of TALE subunits, each of them having the capability of recognizing a specific DNA nucleotide chain independent from other, this result increase in number of target sites for nucleases enzyme with high precision.[4] CRISPER nucleases have a slightly lower precision when compared to TALE nuclease. This is caused by the need of having a specific nucleotide at one end in order to produce the guide RNA that CRISPER uses to repair the double strand break it induces.[6,9]

APPLICATIONS OF GENOME EDITING IN CROP IMPROVEMENT

Before the emergence of engineered nucleases, genetic modification was labourious, intensive, and costly method,[11] However, with the advent of economic and user-friendly gene-editing technologies, custom cell lines carrying nearly any genomic modification can now be generated in simply a matter of weeks. Examples of some of the outcomes that have become routine because of the emergence of targeted nucleases include gene knockout (gene deletion, gene inversion, gene correction, gene addition and even chromosomal translocation.[12] Both TALENs and CRISPR-Cas9 have been used to modify multiple alleles within hexaploid bread wheat to confer heritable resistance to powdery mildew.[5,8] Some data also showed that TALENs has been used to knock out nonessential genes in the fatty acid metabolic pathway in soybean plants and was found to be effective to generate a simplified plant cell having less number of metabolic components and hence increase life. For intense, two recent reports showed that purified nuclease proteins can be introduced directly into plant protoplasts.[13] Finally, targeted nucleases have also been used to inactivate pathogenic genes to prevent viral (or parasitic infection, as well as to introduce knocking-specific factors capable of imparting pathogen resistance.[14]

CONCLUSION

Agricultural biotechnology includes a number of scientific techniques which are commonly used to improve plants, animals and microorganisms to achieve crop yields. The above described method demonstrated that genome editing processes are though not easy but are cost-effective and user-friendly and can be used for variety of plant species. The same may be effectively helpful to fulfill the demand of food with the growing population.

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