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CRISPR-Cas Technology in Agriculture Medicine and Bio-energy

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Introduction

One of the greatest innovations in the field of biology in 21st century is establishment of Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) and its associated protein Cas as a genome editing tool. CRISPR and its associated technologies have multitude of applications. Application of this technology comes in handy when solving 21st problems related to hunger and food security, human healthcare and solving energy needs. CRISPR-Cas technologies have increased yield and quality of food, helped in plant and human disease diagnosis and increased biofuel production in algal system. Multidimensional aspect (Fig. 1) of this revolutionary technology has been discussed below.

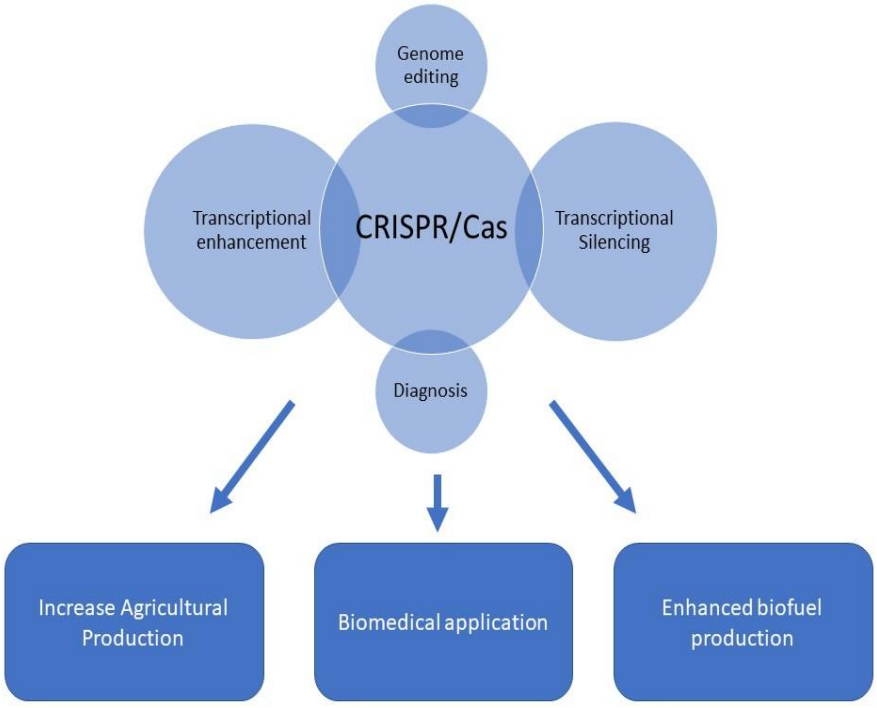

Figure 1. Multi-dimensional application of CRISPR/Cas technology

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CRISPR/Cas in Crop Improvement

CRISPR-Cas has now been used widely in yield and quality improvement and disease resistance of crop plants. Yield related genes, *Gn1a* for grain number,GS3 for grain size,DEP1 for panicle size and IPA1 for tillering and plant architecture has been muted in a Japonica rice cultivar by CRISPR/Cas9 which yielded more grain per panicle, larger grain size and erect panicle (Li et al., 2016).Three major negative regulator genes of rice grain weight GW2, GW5 and TGW6 were simultaneously knocked out using the multiplex CRISPR/Cas9 system and up to 29% increase in thousand grain weight (TGW) has been obtained successfully (Xu et al., 2016). CRISPR/Cas9 has also been used to create homozygous wheat mutant for TaCKX2 gene, a negative regulator for grain number and resulted in increased grain production per spikelet (Zhang et al., 2019). ENO, an AP2/ERF super family of transcription factor, regulates SlWUS expression to restrict stem-cell proliferation in flower to maintain fruit size. CRISPR/Cas9 mediated mutation in ENO gene produce more multilocular larger fruits due to an expanded floral meristem (Yuste-Lisbona et al., 2020).

Crop quality is also very important for agricultural production. One of the major desirable properties in starch producing crops is waxy phenotype, in which loss of function of Granule bound starch synthase-I (GBSS-I) gene results in starch with amylopectin only. Transient expression of CRISPR-Cas9 and sgRNA in potato protoplast successfully created trans-gene free multiallelic GBSS-I mutant lines with high amylopectin content in the tuber (Andersson et al., 2017). Development ofgenome edited superior waxy corn hybrids in 12 elite inbred maize lines has been reported by bombardment of different vectors containing CRISPR–Cas9, sgRNA, selectable marker and morphogenic genes and thereby generation of lines with two waxy deletion alleles without transgene in segregating population (Gao et al., 2020).Low-gluten wheat lines with up to 85% reduction of immunoreactivity have been developed using CRISPR-Cas9by targeting the α-gliadin gene (Sánchez- León et al.,2018).Additionally, CRISPR-Cas has made it easier to develop superior crops with higher levels of oleic acid (Do et al, 2019), lower phytic acid (Khan et al., 2019)and increased carotenoid content (Dong et al., 2020).

CRISPR-Cas in disease resistance and diagnosis of plant

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CRISPR-Cas system has been widely used to tackle plant pathogens. Rice lines with broad spectrum resistance against blast disease causing pathogens Xanthomonas oryzaepv. oryzae has been created with help of CRISPR-Cas by creating mutations in the promoter region of SWEET11,SWEET13 andSWEET14 susceptibility genes (Oliva et al., 2019).

De novo domestication of plant

CRISPR-Cas has accelerated de-novo domestication of new plant species. Multiplexed CRISPR–Cas9 system has been used to edit domestication related traits in wild tomato species *Solanum pimpinellifolium*. Simultaneous editing ofmultiple genes, SP (plantgrowth habit), OVATE (fruit shape), FW2.2 (fruit weight) and CycB (lycopene content)(Zsögön et al., 2018); MULT (fruit number),CLV3 and WUS(fruit size), OVATE (fruit shape), SP (plant growth habit), SP5G (floral induction),GGP1 (vitamin C content) and CycB (lycopene content) (Li et al., 2018) has advanced *S.pimpinellifolium* toward becoming a desirable tomato variety.

Medicinal use of CRISPR/Cas technology

Repairing genetic disease-causing mutations is one of genome editing's most evident therapeutic uses, there are several editing techniques that may change genes implicated in more widespread, complex disorders (Pickar-Oliver & Gersbach,2019). Adenovirus mediated delivery of CRISPR-Cas9 in mouse liver, knocking down PCSK9 gene significantly increased hepatic low-density lipoprotein receptor levels and reduced blood cholesterol level (Ding et al., 2014). The CRISPR-Cas systems were created to be efficient and precise SARS-CoV-2 diagnosis tools (Zhu et al., 2021).DETECTR, a SARS-CoV-2 detection assay has been developed, which utilizes reverse transcription and loop-mediated amplification (RT-LAMP) alongwith Cas12a ssDNA cleavage property to cleave a reporter molecule confirming detection of the virus (Broughton et al., 2020). CRISPR-Cas13a, which cleaves RNA has been used to target EGFP mRNA expression in human glioma cells, the CRISPR-Cas13a system also caused collateral RNA breakage in U87-EGFRvIII glioma cells. Additionally, in an intracranial glioma tumour model, the CRISPR-Cas13a system was discovered to successfully stop tumour growth and angiogenesis. CRISPR-Cas9 gene editing provides a powerful tool to enhance the natural ability of human T cells to fight cancer. The safety and viability of multiplexed CRISPR-Cas9 editing to design T cells in patients with refractory cancer were tested in a first-in-human phase 1 clinical trial. To lessen TCR

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mispairing and improve the expression of a synthetic, cancer-specific TCR transgene, two genes encoding the endogenous T cell receptor (TCR) chains, TCR-α and TCR-β, were eliminated in T cells (NY-ESO-1). To enhance antitumor immunity, a third gene that encodes programmed cell death protein 1 (PD-1) was removed. A lasting engraftment with modifications at all three genomic loci was produced by adoptively transferring modified T cells into patients. For up to nine months, modified T cells persisted and proved effectiveness of CRISPR gene editing in cancer immunotherapy(Stadtmauer et al., 2020).

CRISPR/Cas in biofuel

CRISPR-Cas systems have immense potential to improve biofuel production in microbes by inhibition of competitive pathways, redistribution of metabolic flux and improve substrate utilization to solve our energy crisis (Shanmugam et al., 2020).Lipid production in microalgae *Nannochloropsis gaditana*has been doubled by CRISPR-Cas9 mediated knock-out of a transcription factor, homolog of fungal $Zn(II)_2Cys_6$, which is a negative regulator of carbon partitioning for lipid production (Ajjawi et al., 2017). In another study, *Clostridium tyrobutyricum* has been successfully modified using endogenous CRISPR-Cas system to produce butanol efficiently, by adding *adhE2* (aldehyde/alcohol dehydrogenase gene from *C. acetobutylicum*) while simultaneously removing*cat1*(butyrate: acetate CoA transferase) gene, the resulting mutant produced a record-breaking 26.2 g/L of butanol (Zhang et al., 2018).

Conclusion

CRISPR/Cas systems have offered a versatile, user-friendly molecular platform for accurately altering and regulating the genomes of organisms across a wide range of sectors, speeding up genetic research and the development of gene therapy for the treatment and curing of disease. Life-threatening disorders, such as cancer, cystic fibrosis, DMD, TDT, SCD, and EB, have been shown to be treatable with CRISPR-Cas systems by editing the genes linked to the disease with minimal off-target consequences. CRISPR-Cas technologies in agriculture have shown their potential to boost agricultural productivity and quality, improve crop resistance to drought, herbicides, and insecticides, and lengthen the shelf life of vegetables, all of which improve food safety. *Clostridium*, *E. coli*, *B. subtilis*, and other microbes' genomes have already been edited effectively using CRISPR-Cas-based genome editing tools. CRISPR/Cas technology will surely bring more prosperity in the world.

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