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CRISPR: A Breakthrough in Crop Improvement

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Abstract

(CRISPR-Cas9) genome editing system has been identified as one of the most useful biological tools in genetic engineering ensuring global food security. Some earlier gene editing methods such as meganucleases (MNs), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) allowed site directed modification but their success rate was limited. In this context, the clustered regularly interspaced short palindromic repeat-Cas (CRISPR/Cas) based gene editing tool has revolutionized the field of agriculture due to its simplicity, efficiency, accessibility, reproducibility and adaptability. In this article, we summarize the latest findings on understanding the mechanism of abiotic and biotic stress management in plants and the application of CRISPR/Cas-mediated gene-editing system towards enhancing yield and quality of various crops. Even though CRISPR/Cas-9 ushers in a new age of molecular biology and has a multitude of uses from fundamental molecular research to therapeutic applications, there are still limitations that need to be resolved before practical applications.

Introduction

Global food security system is under jeopardy as the agricultural production is strongly affected by climate changes and pathogen attack. Looking forward, major challenges of the 21st century is meeting the nutritional requirements while combating the climatic changes (Hemalatha et al., 2023). Even though conventional breeding methods have made a significant contribution to the development of abiotic stress tolerance in crops, however they are costly and time consuming. Hence, new technologies must be implemented quickly to offer crop cultivars with stress adaption traits in the future to achieve the anticipated increases in agricultural yields (Manavalan et al., 2009). Genome editing tools are technologies used to modify DNA sequences within an organism's genome. They have revolutionized biological research and hold great potential for various applications in fields like medicine, agriculture, and biotechnology. Some of the prominent genome editing tools includes meganucleases, transcription activator-like nucleases (TALENs), zinc-finger nucleases (ZFNs) and CRISPR (Asmamaw and Zawdie, 2021). The novel application of the CRISPR-Cas tool is its capacity to start genetic modifications at the desired specific DNA target sites and modify DNA nucleotide sequences by editing nucleotide bases or changing the structure of chromatin assembly, leading to epigenetic changes, rather than creating disruptions in double-stranded DNA (Redman et al., 2016).

In 2012, Doudna, J., and Charpentier, E. demonstrated that, with the correct template, CRISPR/Cas-9 could be used to modify any desired DNA. Since then, CRISPR/Cas-9 has emerged as the most precise, effective, and efficient technology for editing the genome in all living cells (Adli, 2018; Jinek et al., 2012). The two main components of the CRISPR/Cas9 gene-editing technique are Cas9 (CRISPR-associated protein 9), an endonuclease that breaks double strands of DNA to allow alterations to the genome, and a guide RNA that matches a particular target gene (Mei et al., 2016). Extracted from Streptococcus pyogenes (SpCas-9), the Cas-9 protein was the first Cas protein employed in genome editing. Prokaryotes employ CRISPR and its associated protein (Cas-9) as an adaptive immunity mechanism for shielding themselves against viruses or bacteriophages (Hille and Charpentier, 2016). The three basic stages of CRISPR defence mechanism against viral attack includes: adaptation (spacer acquisition), crRNA synthesis (expression), and target interference (Rath et al., 2015).

Applications of CRISPR Technology

Abiotic stress management in crop plants

Drought, salinity, cold, heat, and heavy metals are some of the abiotic factors that significantly reduces agricultural productivity across the globe. These factors hinder the plants' ability of growth and development, thereby affecting their yield and quality.

Plants shows changes at morpho-physiological, biochemical and molecular levels in response to abiotic stress (Boyer, 1982; Pandey et al., 2017). CRISPR Cas targets stress sensitive genes associated with regulatory networks, metabolic pathways and signal transduction thereby impacting crop productivity and enabling them to endure unfavourable environmental conditions. CRISPR edited plants show enhanced photosynthetic rate, biomass, nutrient content, chlorophyll content while reducing transpiration, metal toxicity, and oxidation products in abiotic stress tolerant plants (Liao et al., 2019). CRISPR mediated gene editing has been done for several genes such as aldehyde dehydrogenase, mitogen-activated protein kinase, ethylene responsible factor, abscisic acid activated protein kinase 2, and phytoene desaturase for improving abiotic stress tolerance in plants. For example, in tomatoes, knockout of SlMAPK3 enhances heat tolerance through reactive oxygen species (ROS) homeostasis. Similarly, the activation of vacuolar H+-pyrophosphatase (AVP1) in *Arabidopsis* increases leaf surface area and increased resilience to the effects of drought (Lou et al., 2017; Debbarma et al., 2019). CRISPR/Cas9 system has been applied in several other plant species such as *Brassica napus, Oryza sativa, Cicer arietinum* and *Glycine max* (drought tolerant); *Medicago Truncatula* and *Solanum lycopersicum* (salt tolerant); *Gossypium hirsutum* and *Lactuca sativa* (heat tolerant); *Zea mays* and *Saccharum officinarum* (herbicide resistant) (Kumar et al., 2023).

Disease resistance in plants

Plant diseases resulting from bacterial, fungal, and viral pathogens cause significant losses in agricultural productivity. The CRISPR-Cas9 system provides an accurate approach to overcome several agricultural challenges including fungal, bacterial and viral resistance in various plant species. Geminivirus genomes, formed by ssDNA are the main target of CRISPR-cas9 modifications. Several economically significant crops from different families, such as Solanaceae, Fabaceae, Cucurbitaceae, Euphorbiaceae, Malvaceae, and Geminiviridae, are infected by *Geminiviridae* (Zaidi et al., 2016). For example, CRISPR-Cas 9 induced resistance to geminiviruses, beet severe curly top virus and bean yellow dwarf virus in *Arabidopsis* and Benth, respectively. Mutagenesis in banana plant against banana streak virus led to resistance to 'banana streak' disease. In addition to being asymptomatic, phytopathogenic bacteria are more difficult to control than viruses and fungi since there aren't enough chemicals available. CRISPR/Cas9-edited citrus plants showed resistance to *Xanthomonas citri*, the causative agent of citrus bacterial canker (CBC) disease; mutagenesis of the (*ethylene responsive factor*) ERF Transcription Factor Gene in rice provides resistance against blast; fire blight resistance in apple and bacterial speck disease resistance in banana (Boubakri et al., 2023). CRISPR technology has also been used to strengthen crops' defences against parasitic plants, however this field is still in its early stage. For example, CRISPR edited sunflower varieties produce toxic coumarins that inhibits *Orobanche* development; sorghum plant disrupts the haustorium formation of *Striga asiatica* by releasing inhibitory compounds through roots, gene knockout in tomato provides resistance against the root parasitic weed *Phelipanche aegyptiaca* and sorghum resistance against Striga (Jhu et al., 2023).

Nutritional improvement

Malnutrition is a prevalent concern with the challenge of food security for the world's population. Malnutrition includes deficiencies in vitamins, minerals and other nutrients required for healthy growth, development, and functioning of the body (Kurmi et al., 2023). Crops can be fortified using agronomic methods (use of mineral fertilizers) or breeding (Adu et al., 2018). However, the plant breeding method is laborious and time consuming, on the other hand crops altered through transgenic technology (genetically modified) show allergic reaction and humans and also reduced nutrition. In this regard genome editing (GE) technology offers the advantages of predictable and inheritable mutation. CRISPR is assisting in the improvement of crop quality traits such as desired appearance, nutritional content and palatability (Liu et al., 2021). For example, carotenoid biofortified Kitaake cultivar of rice developed by knock-in *CrtI* and maize *PSY* genes; other example includes tomato, golden banana and golden flesh melon. Tocopherols and tocotrienols enhancement in barley by over expression of phytyltransferase and geranylgeranyl transferase genes. CRISPR-Cas system disrupts *Inositol Pentakisphosphate 2-kinase 1 (TaIPK1)* in wheat, reducing phytic acid content to improve zinc bioavailability in wheat grains (Kumar et al., 2022).

Limitations

Despite the promising benefits of CRISPR in plant science, off target effects remains a major challenge that may lead to adverse outcomes. The off-target effects are frequently sgRNA-dependent but some sgRNA-independent off-target effects also exist. Requirement of PAM (Protospacer adjacent motif) near target site is another drawback. Despite its widespread use, Cas 9 from Streptococcus bacteria (SpCas9) is challenging to incorporate into AAV due to its huge size (Lino et al., 2018). Achieving high efficiency and specificity of genome editing remains a challenge in certain plant species and tissues. Plant genomes can be complex, with polyploidy, gene redundancy, and functional redundancy posing challenges for precise genome editing. Targeting multiple genes or regulatory elements simultaneously may be necessary to achieve desired traits, further complicating the editing process. CRISPR-mediated genome editing can sometimes induce unintended changes in epigenetic regulation, altering gene expression patterns even without altering the DNA sequence (Uddin et al., 2020). Also, intellectual property rights associated with CRISPR technology and specific gene targets may limit access to CRISPR tools and technologies for researchers and breeders.

Conclusion

CRISPR technology is versatile and has transformative potential across diverse fields thereby it has surpassed other methods. CRISPR based gene editing has revolutionized the field of agriculture by developing nutritionally enhanced crops and those resistant to bacteria, virus, fungus and resistant to parasitic plants thereby addressing the issue of food security. Genetic modification using CRISPR can render plants resistance to unpredictable climate conditions (biotic and abiotic stress). Although CRISPR/Cas-9 technology holds immense potential as a genome-editing method, still there are several challenges that need to be addressed during the process of application.

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