Review paper

Use of CRISPR-Cas9 in agriculture

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Abstract
The agricultural industry faces numerous challenges, such as climate change, biodiversity loss, water shortage, extensive land usage, chemical fertilizers, and food waste. CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) technology has emerged as a potential solution to address these challenges. By altering crops' genomes, this technology can produce desirable traits like increased protein or starch content, drought resistance, heat tolerance, viral or bacterial resistance, increased yield, increased and mite resistant bee pollinator populations, weather-time synchronized flowering and fruiting, improved shelf life of harvested fruits and seeds, modifying alkaloid biosynthesis and increased carbon capture from the environment. Moreover, CRISPR/Cas9 can enhance the nutritional value of crops by targeting specific genes that control the production of bioactive compounds like phenolics, carotenoids, vitamin E, dietary fiber, and beta-glucan. This review discusses the current challenges in agriculture and highlights the potential solutions that CRISPR/Cas9 technology offers to overcome these challenges.

Keywords
Agriculture, Climate change, CRISPR-Cas9, Drought resistance, Heat tolerance, Yield, Flowering, Cabon capture, Nutritional value, Pollination

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Introduction

The limitations of chemical intensive agriculture and the role of CRISPR-Cas9

The agricultural industry faces challenges of biodiversity and habitat loss, climate change, water scarcity, disproportionately extensive land usage, indiscriminate use of chemical fertilizers and insecticides, and waste of produce. While agriculture does play an essential role in addressing food insecurity, some challenges and barriers affect its ability to provide food security adequately. Food insecurity is still a looming crisis threatening millions worldwide due to population growth, urbanization, poverty, economic instability, and conflict (1).

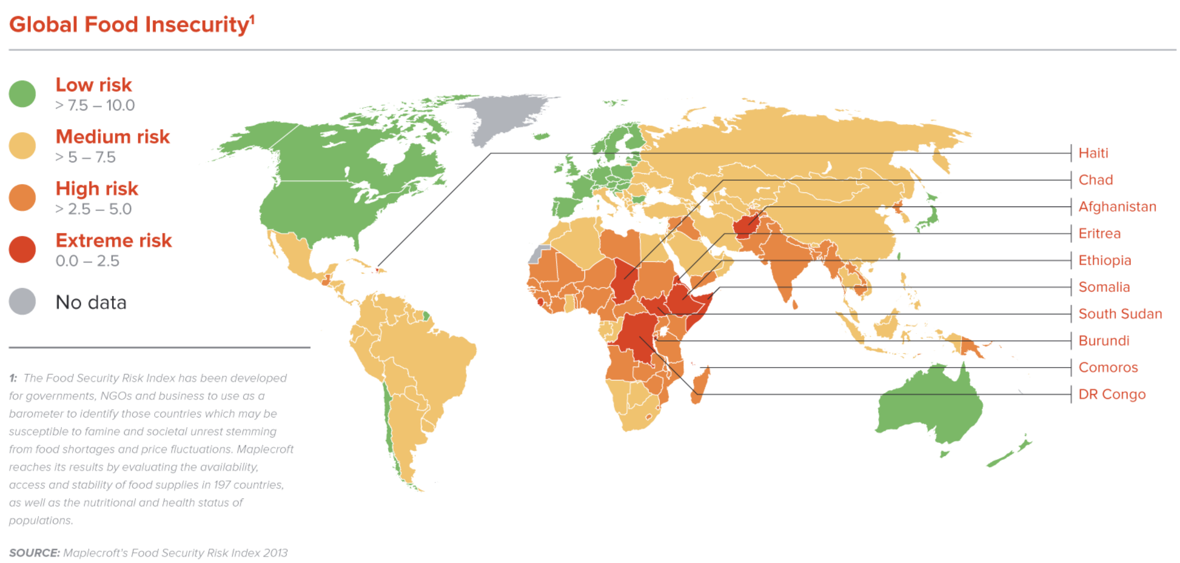

Figure 1. The map provides a visualization of the extent to which food insecurity affects the world (51).

CRISPR/Cas 9 has the potential to improve food security by increasing crop yield and quality, enhancing nutritional value through biofortification, and reducing post-harvest produce losses. This gene-editing tool can also mitigate greenhouse gas accumulation by increasing carbon capture.

Climate change is a major challenge for farmers, affecting crop productivity, water availability, pest and disease outbreaks, and soil quality (2). The release of greenhouse gases and climate change affect agricultural producers significantly because agriculture depends on specific climactic conditions. Dramatic temperature changes can cause habitat ranges and crop planting dates to shift, and droughts and floods due to climate change may hinder farming practices (3). They can also have a detrimental impact on wildlife and pollinators. CRISPR/Cas 9 can help farmers adapt to climate change by making crops more tolerant to extreme climactic conditions such as drought, heat, salinity, flooding, or cold (4).

Agriculture can modify biodiversity in several ways. One of them is by converting natural habitats into intensely managed systems for farming, which results in habitat loss for many species and a decline in biodiversity. Additionally, agricultural practices can release pollutants such as greenhouse gases. CRISPR-Cas9 can mitigate some environmental issues associated with agriculture by improving crop traits such as resistance to pathogens and/or pests. This can reduce the need for harmful pesticides and herbicides that would otherwise cause eutrophication by polluting waterways, forming toxic algal blooms and contributing to aquatic ‘dead zones’. Additionally, CRISPR-Cas9 can be used to make plants more resistant to environmental stresses such as poor weather conditions, which would help increase yields at the end of the season and reduce the need for resource/chemical intensive farming practices. A notable example of a crop that is being enhanced with such traits is rice, which has been found by researchers to be a cost-effective and sustainable approach (5).

CRISPR Cas9

The original biological function of CRISPR–Cas9 system is the protection of prokaryotes from viruses. This system can be used as a tool to find specific DNA sequences and replace them with desired ones. Cas9 (or “CRISPR-associated protein 9”) is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence. In other words, the Cas9 protein enzyme acts as a DNA scissor. Its role is to cut out or replace certain strands of DNA. One of the most important uses for CRISPR-Cas9 is that it can be used as a gene-editing tool, as it can snip out and/or replace DNA strands (6-13).

Very simply, the process of using the gene-editing tool begins with designing an appropriate guide RNA that will direct Cas9 to the desired location in the genome. Then the Cas9 enzyme is transported by the RNA into the target cell's nucleus, where it is subsequently guided by an artificially constructed guide sequence to find a matching set of DNA to snip. Once the guide RNA locates the target, it cleaves specific strands that match its sequence by creating double-strand breaks in the DNA. After the Cas9 enzyme cuts out that certain strand of DNA, it needs to repair itself with a new set of DNA strands. In this case, the new set of strands are the desired and modified sequences. The CRISPR modified cells then express (the product of) the modified/inserted gene and/or do not express the (product) of the detrimental gene. While the technology has evolved, it is a work in progress and still relies on largely empirical protocols to obtain the desired gene expression and reduce ‘off-target” effects.

Discussion

Modification of nutritional content

CRISPR-Cas9 can potentially modify the nutritional contents of crops, such as seed grain weight, protein content, polyunsaturated fatty acid content, mineral content, and amylopectin content. In this application, the CRISPR-Cas9 genome-editing tool differs from other genetically modified crops in that it does not introduce foreign genes into the plant's genome. Instead, it targets specific genes responsible for nutrient biosynthesis and modifies them to enhance their nutritional value. Specifically, it targets specific genes that control the production of bioactive compounds such as phenolics, carotenoids, vitamin E, dietary fiber, and beta-glucan. By manipulating these genes, researchers can increase the production of these compounds in crops and thereby improve their nutritional value (14). This approach has been successfully used to biofortify cereal and vegetable crops including rice, wheat, and carrots. CRISPR Cas9 has also been used to enhance the vitamin A content of tomatoes as well as the beta-carotene content of carrots (14). In one study, CRISPR-Cas9 was used to target the FAD2 gene in *Camelina sativa* to improve seed oil composition. There was a 16% to 50% increase in the oleic acid content of the Camelina seeds (15).

Iron is an essential micronutrient for plant growth and development. Rice is a staple food for more than half of the world's population, but it contains low concentrations of iron in polished seeds, which is disadvantageous as iron is an important part of the human diet (16). Understanding the molecular components of iron uptake and translocation as well as their regulation has paved the way to develop crops that are tolerant to iron deficiency, both to improve food and biomass production, as well as to develop iron-rich crops for human consumption. In rice crops, it was found that the genes OsIRT1, OsIRT2, and OsYSL15 play a role in iron uptake from the soil, and the gene OsYSL2 plays a role in phloem mediated iron distribution in plants (16). By using CRISPR-Cas9 to target these specific genes, the iron content of rice can be increased significantly. As iron deficiency is common in the under-nourished, the biofortification of the crop can help to mitigate this deficiency especially in developing countries where rice is a staple food (16).

Table 1. The examples of a select group of CRISPR-Cas9 modified crops, the specific genes modified and their effects on nutritional improvement.

Crop	Nutritional improvement	Gene modified	References
Rice	Increased iron and zinc content	OsYSL2, OsNAS2, LcyE, OsIRT1, OsIRT2, OsYSL15	(14,16,17)
Wheat	Enhanced protein content	Psy1, TaGW2, TaGW7	(14,18,19)
Tomatoes	Enhanced vitamin A content and carotenoid biosynthesis	Psy1, MYB12	(14,20)
Carrot	Increased beta-carotene (provitamin A)	Psy1, LcyE, LcyB, CrtB1, CrtRB2	(14,21)
False flax *(Camelina sativa)*	Increased omega-3 fatty acids	FAD3A, FAD3B	(14)

Researchers have also suggested that the use of CRISPR-Cas9 technology helps to target the genes responsible for melatonin biosynthesis in crops, meaning that the amount of melatonin production can be increased, thereby improving nutritional value. Melatonin is an essential hormone in the human body because it helps to regulate the body’s natural sleep cycle, and thus acts as a natural treatment for people who have insomnia, allowing them to sleep better. It has also been suggested that melatonin can block stomach acid secretion and nitric oxide synthesis. It also helps to reduce heartburn when used alone or with medication (22).

Adaptation to climate change

CRISPR-Cas9 can be used to modify crops and trees to better adapt to harsh climactic conditions such as sweltering and/or arid climates, sudden heavy rainfall and/or a temporal shift of seasons while simultaneously providing good yield. For example, the technology enabled the breeding of maize variants containing the gene expression ARGOS8 (23,24). ARGOS8 is responsible for reducing ethylene sensitivity and improving yield under drought conditions. According to one study, the maize variants that carried the gene expression increased grain yield under flowering stress conditions with no yield loss under well-watered conditions (25,26). In another study, scientists targeted specific genes responsible for regulating drought tolerance in crops, specifically rice and wheat. In rice, the specific gene that was targeted was the DREB1A gene and in wheat, the TaERF3 gene was targeted to improve drought tolerance (14, 27,28). In addition, there was also a study conducted on rice plants that reduced stomatal density, which was found to improve water conservation and drought tolerance (29). The study found that these genetically engineered plants had a lower transpiration rate and higher water use efficiency, making them more resilient to drought conditions. The reduced stomatal density was achieved using CRISPR-Cas9, which targeted a specific gene responsible for stomatal development known as EPFL9 in rice (30). It was found that the expression of this gene was necessary to regulate stomatal density, and that reducing its expression led to a significant decrease in stomatal density. Normally in plants, the stomata serve as means of gas exchange which also exchanges water throughout the process, and the reduction of the stomata led to the observed improvements in water conservation and drought tolerance, as less water would be lost through evapotranspiration (29). Overall, these studies provide promising solutions to the challenge of water usage because crops modified using CRISPR-Cas9 can help to conserve water, which is crucial in arid regions worldwide, such as in California and the Sahel; among others.

Resistance to pests

The CRISPR-Cas9 gene editing system has also been used to address pest control. Some frequent threats to crops include plant diseases which are often caused by viruses, fungi, or bacteria, and thus the engineering of resistance against such pathogens is of great importance for agricultural sustainability. One such example is powdery mildew, which is a fungal disease that affects many crops, including wheat, and can cause significant yield losses. CRISPR-Cas9 was used to simultaneously edit three homo-alleles in wheat, which conferred heritable resistance to powdery mildew. The researchers targeted the *TaMLO* gene, which encodes a protein that is required for powdery mildew to infect wheat. The T7 endonuclease I assay was used to identify mutations induced by sgMLO-A1 in wheat protoplasts and transgenic wheat plants. The rapidity and precision with which changes can be achieved by this approach are expected to improve wheat yields at a rate sufficient to guarantee global food security (31). Another study was conducted on citrus canker disease (a bacterial plant disease which affects citrus trees). In the study experimenting with the effects of the pathogen, CRISPR-Cas9 was used to modify the PthA4 effector binding elements, which induces the CsLOB1 (Citrus sinensis Lateral Organ Boundaries) gene. The activation of a single allele of CsLOB1 by PthA4 was found sufficient to induce citrus canker disease, and mutation in both alleles was found necessary to generate resistance to citrus canker (32). Pyott et al., provided insights into how the CRISPR/Cas9 gene editing system was utilized to create resistance against viral pathogens in plants (33). The researchers focused on potyviruses, a group of RNA viruses that infect a wide range of plant species, including *Arabidopsis thaliana*, which was used as a model plant in this study. The CRISPR/Cas9 system was employed to target and modify specific sequences within the viral genome, aiming to disrupt essential viral functions and render the plants resistant to potyvirus infection. The researchers designed guide RNAs (gRNAs) that specifically targeted conserved regions of the viral genome. These gRNAs guided the Cas9 endonuclease to introduce double-stranded breaks at the targeted sites, triggering DNA repair mechanisms in the plant cells. By introducing the CRISPR/Cas9 system into *Arabidopsis* plants and targeting the genome of a potyvirus, the researchers were able to generate transgene plants that exhibited resistance against potyvirus infection (33). The modified plants showed reduced symptoms of viral infection, including milder leaf lesions and lower viral RNA accumulation compared to the non-edited control plants. Furthermore, the researchers observed stable inheritance of the resistance trait in subsequent generations of the edited plants, demonstrating the heritability of the engineered resistance. This finding was significant as it suggested the potential for durable and long-term protection against potyvirus infections in crops. The study highlighted the potential of the CRISPR/Cas9 gene editing system as a powerful tool for engineering resistance against viral pathogens in plants. By precisely targeting and modifying viral sequences, it is possible to disrupt crucial viral functions and enhance the plant's defense against viral infections. Overall, these studies on the activation of pest resistance in crops illustrate the versatility and efficacy of gene editing technologies in addressing pest and disease control in agriculture. By understanding the molecular mechanisms underlying plant-pathogen interactions and utilizing techniques like CRISPR/Cas9, researchers can develop crops with enhanced resistance to pathogens, ultimately contributing to sustainable and productive agricultural practices (33).

Produce quality and shelf-life

CRISPR-Cas9 is also beneficial to agriculture in that it is used to improve not only the shelf-life of crops but also their overall quality as well. In one study on the tomatoes' shelf-life, CRISPR-Cas9 was used to obtain the tomato ALC gene (34). The desired ALC gene was acquired and further confirmed by genotype characterization and was revealed to be in good condition under storage. Thus, this demonstrates one example of the use of CRISPR-Cas9 on crops to help improve its shelf-life (34-36). In another study, scientists utilized the gene editing tool to improve the shelf life of the melon species *Cucumis melo* (37). A targeted modification of the CmACO1 gene was performed using CRISPR/Cas9 technology. The CmACO1 gene is involved in the ethylene synthesis pathway and is a key gene for regulating the melons' shelf life. Ethylene is a plant hormone that plays a critical role in fruit ripening and senescence. The CmACO1 gene encodes an enzyme called ACC oxidase, which catalyzes the final step of the ethylene biosynthesis pathway. By modifying this gene, ethylene production in the melons was reduced, consequently, the shelf-life of the melons was significantly increased, reducing produce loss and contributing to food security. Compared to conventional breeding methods, genome editing technology offers several advantages for commercial applications, including precision, speed, and efficiency. This has important implications for the future of fruit production, as it could lead to reduced produce waste and the cost of exportation, as well as increased availability of fresh produce. Overall, this study demonstrates the potential of CRISPR/Cas9 technology for improving the sustainability and efficiency of the global food system (37).

Bioenergy from post-extracted plant material

CRISPR-Cas9 was used to increase bioenergy production from sugarcane plants. The recent availability of the sugarcane plant's genome sequence paved the way for utilizing CRISPR-Cas9 in the study of its biomass and bioenergy traits. Through its versatile and cost-effective means of precisely deleting, inserting, or replacing genes, CRISPR-Cas9 was shown to be a promising method for the enhancement of the sugarcane's potential as a biofuel feedstock. More specifically, the challenge of lignin modification was addressed, which is crucial for improving fermentable sugar yields for biofuel production. Through the modification of lignin composition and distribution in sugarcane cell walls, CRISPR-Cas9 can enhance the energy content of sugarcane biomass, including bagasse. Bagasse is a fibrous residue that remains after the sugarcane juice has been extracted. It is used as a potential feedstock for second-generation biofuel production, but its use is currently limited by its high lignin content and poor digestibility. CRISPR-Cas9 technology can be used to modify the genes involved in lignin biosynthesis and other traits relevant to bagasse quality, thereby enhancing its potential as a biofuel feedstock. One approach to evaluating edited plants is to use molecular techniques such as the Polymerase Chain Reaction (PCR) and sequencing to confirm the introduction of the desired mutations. This can be done using genomic DNA extracted from the edited plants, which can be amplified using PCR primers that flank the target site. The resulting PCR products can be sequenced to confirm the presence of the desired mutations. Another approach to evaluating edited plants is to use biochemical assays to measure the impact on bagasse quality. This involves analyzing the cell wall components of the edited plants using techniques such as Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. These techniques can provide detailed information on the chemical composition of the cell wall and can be used to assess the impact of gene editing on lignin biosynthesis and other traits relevant to bagasse quality. In addition to molecular and biochemical assays, edited plants must also be evaluated at the whole-plant level to assess the impact on plant growth, development, and bagasse quality. This involves analyzing the edited plants under various growth conditions to determine their performance relative to unedited plants. For example, edited plants can be grown under different light wavelengths, temperature, and nutrient regimes to assess their growth and bagasse quality. While this technology has shown great promise for enhancing sugarcane's bioenergy potential, further research and development are needed to effectively translate these advancements into practical applications. As the field of genome editing continues to progress, it is essential to address the challenges and regulatory considerations associated with CRISPR-Cas9 in sugarcane improvement, ultimately paving the way for sustainable and efficient bioenergy production (38).

Modifying alkaloid biosynthesis

It has also been found that the CRISPR-Cas9 technology can be utilized to knockout genes involved in the biosynthesis of alkaloids such as cocaine, potentially altering or abrogating this biosynthetic pathway so as to produce non-addictive molecules. By targeting specific genes responsible for the production of alkaloids of abuse, CRISPR-Cas9 can disrupt their synthesis in plants. For example, genes encoding key enzymes in the cocaine biosynthetic pathway could be targeted for knockout, leading to reduced or eliminated cocaine production in the plant. Additionally, CRISPR-Cas9 can be used to introduce mutations that alter the structure or function of enzymes involved in alkaloid biosynthesis, potentially resulting in the production of non-addictive alkaloid derivatives. This approach could offer a novel strategy for producing alkaloids with modified properties, such as reduced addictive potential. By precisely editing the plant genome using CRISPR-Cas9, researchers can engineer plants to produce alkaloids that retain their beneficial properties while minimizing their addictive effects. Furthermore, CRISPR-Cas9 technology allows for targeted modifications in specific biosynthetic pathways, offering a tailored approach to alkaloid production. The ability to fine-tune the biosynthesis of alkaloids through CRISPR-Cas9 editing opens up possibilities for developing safer and more controlled production of these compounds (39).

Increasing the population of pollinator Bees and pollination

The use of CRISPR-Cas9 in agriculture is not only limited to the plants themselves, but also its impact on important pollinators such as bees is being investigated as well. Specifically, the gene editing tool is being used to investigate the role of specific genes in the response of honeybees to pollen consumption and Varroa infestation. A study conducted by a team of researchers from the Department of Agricultural, Food, Environmental and Animal Sciences (DI4A) at the University of Udine utilized CRISPR-Cas9 to knockdown the expression of two key genes, vitellogenin (Vg) and juvenile hormone receptor (JHr), in honeybees to study their impact on the bees' behavioral maturation and immune function. The researchers employed CRISPR-Cas9 to generate Vg and JHr knockdown bees by introducing the CRISPR-Cas9 ribonucleoprotein complex into honeybee embryos. This gene-editing technique allowed them to specifically target and reduce the expression of Vg and JHr genes in the bees, enabling the investigation of the genes' functions in response to pollen and Varroa infestation. By analyzing the knockdown efficiency of Vg and JHr using quantitative PCR (qPCR), the authors were able to confirm the successful suppression of these genes in the honeybees. This precise gene-editing approach provided insights into the specific roles of Vg and JHr in the bees' physiological responses to dietary pollen and Varroa infestation. The study further utilized CRISPR-Cas9 to assess the impact of Vg and JHr knockdown on the survival of Varroa-infested bees. The results indicated differential effects of Vg and JHr knockdown on bee survival rates, highlighting the importance of these genes in the bees' ability to resist Varroa infestation. This study demonstrates the utility of CRISPR-Cas9 as a tool for investigating gene function and understanding the complex interactions between genes, diet, and parasite infestation in honeybee health. This is useful in the field of agriculture as it provides important insight into the behavior of bees, which are responsible for the pollination of majority of food crops (40).

Colony collapse disorder is mitigated by sunflowers because sunflower pollen decreases infestation of bees by Varroa mites (40,41). It is tantalizing to speculate that CRISPR-Cas9 can be used to engineer other plants to manufacture pollen with a similar nutritional composition of sunflower pollen. This represents an as yet unexplored fascinating aspect of CRISPR which enables genetically engineering flowering plants to increase bee population and enhance bee-induced fertilization, by secreting ‘Varroa resistant’ pollen.

Improving crop yields by modulating gene expression

CRISPR-Cas9 also has the capability to improve crop yield, for example in rice grains. One study involving Cas9 demonstrated that two genes: Gn1a and GS3, play a role in the size and number of rice grains. The Gn1a allele is responsible for heavy panicles through increased grain amount, whereas GS3 is associated with grain size and weight (42). Further studies found that the combination of loss-of-function mutations in both Gn1a and GS3 resulted in heavy panicles with many large grains (42,43). Yet another study found that the editing of the OsSPL16 gene led to an improvement in rice grain yield. Additionally, two mutants generated in rice cultivars by Cas9: OsAAP6 and OsAAP10, showed an improvement in the yield and quality of the rice. From the studies, it can be concluded that mutagenesis of amino acid genes leads to better yield in the rice plant (44). Zhu *et al.,* used CRISPR/Cas9 to silence a suite of genes in rice, leading to a variety that yielded 31% more grain (45). In Chinese field trials, CRISPR rice increased grain yield by 25% in part by allowing for the PYL genes (PYL1-PYL6 and PYL12 in group 1 and PYL7-PYL11 and PYL13 in group 2) to be silenced. Silencing these genes, created a variety of rice that produced more grain yield than traditional breeding methods such as phenotypic selection or hybridization (45). Grain quality improvement is a key target for rice breeders, along with yield. It is a multigenic trait that is simultaneously influenced by many factors. The use of CRISPR/Cas9 technology provides an opportunity for researchers to improve rice grain quality and yield (46-52).

Increasing crop yields by modulating temporal flowering times

CRISPR also improved crop yield by modulating the flowering time of plants. Flowering time is a critical trait that determines the reproductive success of plants. Premature or late mature flowering can have a significant effect on crop yield and quality. The CRISPR system can be used to target genes that regulate flowering time in plants. By introducing mutations into these genes, researchers can modulate the timing of flowering. For example, the FT gene is a key regulator of flowering time in many plants. By using CRISPR to knock out the FT gene, researchers have been able to delay flowering in several plant species. Similarly, by introducing mutations into other genes that regulate flowering time, researchers have been able to modulate the timing of flowering in a variety of plant species. CRISPR can also be used to introduce new traits into plants that affect flowering time. For example, researchers have used CRISPR to introduce genes from other plant species that promote early flowering in soybeans. This has the potential to significantly increase crop yields in regions with short growing seasons. CRISPR can also be used to study the genetic basis of flowering time in plants. By introducing mutations into genes that regulate flowering time, researchers can better understand how these genes function. This knowledge can be used to develop new strategies for modulating flowering time in plants (53). CRISPR Cas9 gene editing was used to breed new pineapple varieties with resistance to premature flowering. In this project, the researchers identified the genes responsible for premature flowering in pineapples and used CRISPR/Cas9 to modify/silence them. By doing so, they hoped to develop new pineapple varieties that resisted premature flowering and increased production. One of the advantages of using CRISPR/Cas9 in this project is that it is a non-GM approach. This means that the new pineapple varieties developed using this technology are not considered genetically modified organisms (GMOs). This is important because many consumers are skeptical of GMOs, and some countries have strict regulations on their use. The precision of CRISPR-Cas9 is crucial in developing new pineapple varieties with resistance to premature flowering, as it ensures that other desirable traits are not lost in the process. Overall, the use of CRISPR/Cas9 in this project has the potential to revolutionize pineapple farming by developing new varieties that can resist premature flowering. It is hoped that this will increase production and profitability for farmers, benefiting the Australian pineapple industry as well as international markets (54). Furthermore, the non-GM approach used in this project may alleviate concerns about GMOs and make the new pineapple varieties more acceptable to consumers.

Increasing carbon capture and storage

In addition to editing crop genomes to better suit the changing environment, it has also been found that trees can be edited using CRISPR for increased carbon capture and storage. For instance, researchers have successfully used CRISPR/Cas9 to reduce the amount of lignin, a complex organic polymer that interferes with the processing of wood into bio-based products. By targeting both copies of a gene crucial for the biosynthesis of lignin, researchers were able to achieve a stable 10% reduction in lignin amount without affecting the tree’s growth (55,56). A notable initiative in this use of CRISPR-Cas9 is Living Carbon, which is a company focused on rebalancing the planet’s carbon cycle using the power of plants. The company generated high-quality carbon removal projects in the U.S. with true additionality, limited leakage, and unique co-benefits. They used advanced biotechnology, including CRISPR/Cas9, to create seedlings that are unique in their ability to capture more carbon using less land. These biotech seedlings were specifically designed and engineered for these carbon projects (57). One of the key advantages of using CRISPR/Cas9 in this context is its ability to introduce specific changes in the DNA of highly productive tree varieties in a fraction of the time it would take using classical breeding strategies. The mutations introduced through CRISPR/Cas9 are similar to those that spontaneously arise in nature, making the system a versatile new breeding tool to improve agricultural productivity (55).

Future Perspectives

The CRISPR-Cas9 system is a promising tool for improving plant agronomic traits through point mutations, knockout, and single-base editing. It can enhance crop yield, disease tolerance, regulation of gene expression, combat biotic and abiotic stresses, and generate genome-wide mutant libraries (45). Recent developments and up-gradation of delivery mechanisms (nanotechnology and virus particle-based delivery systems) have also been made to facilitate crop domestication and hybrid breeding (58). However, there are still challenges associated with using CRISPR-Cas9 in agriculture. These include regulatory hurdles such as obtaining government approval for genetically modified crops. Public acceptance of genetically modified crops is also a challenge, as some people may have concerns about their safety or environmental impact. Off-target effects refer to unintended changes to the genome that can occur during the editing process. While these effects can often be minimized through careful design of the editing process, there are still potential risks such as unintended gene alterations or environmental concerns regarding the unintentional gene flow into wildlife.

Conclusion

CRISPR-Cas9 technology is an important tool in agriculture, enabling the precise editing of crop genomes to improve desirable traits, such as increased protein or starch content, drought resistance, heat tolerance, viral or bacterial resistance, increased yield, increased and mite resistant bee pollinator populations, weather-time synchronized flowering and fruiting, improved shelf life of harvested fruits and seeds, modifying alkaloid biosynthesis and increased carbon capture from the environment. This technology is valuable for addressing the agricultural industry's challenges, including climate change, land use, and food waste. CRISPR-Cas9 technology can promote sustainable agriculture by reducing the need for chemical pesticides and fertilizers, conserving resources, and minimizing waste.

However, it is important to acknowledge that the potential risks associated with CRISPR-Cas9 technology should be carefully considered and addressed. The unintended spread of genetically modified crops could lead to ecological imbalances or unintended effects on non-target organisms. Furthermore, ethical and societal concerns regarding regulating and controlling genetically modified organisms exist. Thus, it is essential to cautiously approach the use of CRISPR-Cas9 in agriculture to minimize any potential negative effects on the environment and human health. The responsible use of CRISPR-Cas9 technology in agriculture can help achieve more sustainable, efficient, productive and equitable agricultural practices, benefiting farmers and consumers.

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