



Advancing Quality and Productivity in Floricultural Crops through CRISPR/Cas9 Technology

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Abstract

Worldwide demand for flowers is rising with time. Today, floriculture is the most important agricultural commodity traded commercially. While the formation of remarkable flower varieties has been greatly aided by conventional breeding procedures like hybridization and mutation breeding, there is still potential for substantial advancement in flower productivity as well as quality. Producers and consumers are seeking novel qualities that are of significance through modern breeding techniques. Through genetic modification, traits like fragrance, distinct blossom colour, altered flower structure and shape, vase life, scent, and capacity to withstand both biotic and abiotic stresses have been developed. The CRISPR- (Cas) protein system, which consists of short palindromic repetitions that are grouped and are regularly spaced, has developed into a powerful genome-editing instrument to precisely alter genetic encodings at designated sites. It serves as an excellent way of enhancing floriculture crops genetically. CRISPR-Cas9 is crucial for agricultural crops because it may improve blooming traits including colour modification, prolong flower shelf life, encourage the emergence and maturation of flowers, and use techniques for genome editing to change desirable foliage's colour. Among many other advantageous traits, Cas9/CRISPR genome editing is helpful in creating new varieties with improved fragrance and essential oils (Sirohi et al. 2022). A CRISPR/Cas tool that can separate from the Cas9/gRNA construct to prevent comparable changes by CRISPR/Cas generates stable gene mutations. CRISPR/Cas gene is a rapid and precise genetically engineered crop technology that produces crops resistant to abiotic and biotic stresses, viruses, fungus and bacteria in a fraction of the time compared to crops generated using conventional methods, which take ten to fifteen years to achieve resistance. Thus, CRISPR/Cas is a helpful tool for producing agricultural products in a sustainable manner. This technology has been used to successfully modify plant characteristics. This has been used to successfully modify plant characteristics.

Keywords: CRISPR/Cas, Genome Editing, Genetic Engineering, Ornamental Crops

INTRODUCTION

The scientific community is quite excited about the new DNA editing technology CRISPR-Cas9. It is cheaper, faster, better than earlier DNA modification strategies and offers a multitude of potential uses. Researchers investigating genetics and medicine may add, remove or alter certain DNA sequences, altering portions of the genome, thanks to a breakthrough method called CRISPR-Cas9. It is now the simplest, straightforward, adaptable and exact genome editing method available. It's generating implications in the field of science. The academic community is quite excited about the CRISPR-Cas9 method because it provides a number of benefits over current genome editing methods, including speed, affordability, precision and efficiency. It is estimated that in the next few decades, there will be 9.8 billion people on the planet, and that the need for food would increase by almost 110% from 2005 levels. The most important and challenging issue at hand is how to mitigate the consequences of global warming while simultaneously ensuring safety of food for the world's expanding population. Global food production needs to be increased immediately, but agriculture is confronted with a number of challenges, including possible environmental risks, the rise of diseases and pests, global variations in temperature and fast population expansion [1]. Remarkable progress in plant genome editing techniques

coupled with the availability of genome sequences for numerous crops has enabled the breeding of any desired characteristic. Recent advances in DNA editing using specific-site nuclease have enabled precise, efficient investigations involving selective transgenic placement, genetic manipulation and reverse genetics. Targeted Double Strand Breaks (DSB) DNA, which promotes repairing genetic mechanisms and are introduced using designed nuclease. Depending on the repair procedure and the accessibility of repair templates, various genetic modifications may be accomplished. Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ) are two different types of repair DSB processes that have been discovered. Additionally, a double-stranded DNA template with precise target implementation is mediated by non-homologous end-joining. The disciplines of biotechnology, basic science, and medicine stand to gain much from the use of biological processes and species engineering. Zinc-Finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENs), and Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology are genome editing techniques which surfaced in recent times [2]. The first two techniques connect DNA-binding proteins with endonuclease catalytic subunits, therefore introducing targeted double-strand DNA breaks (DSBs) at certain sites.

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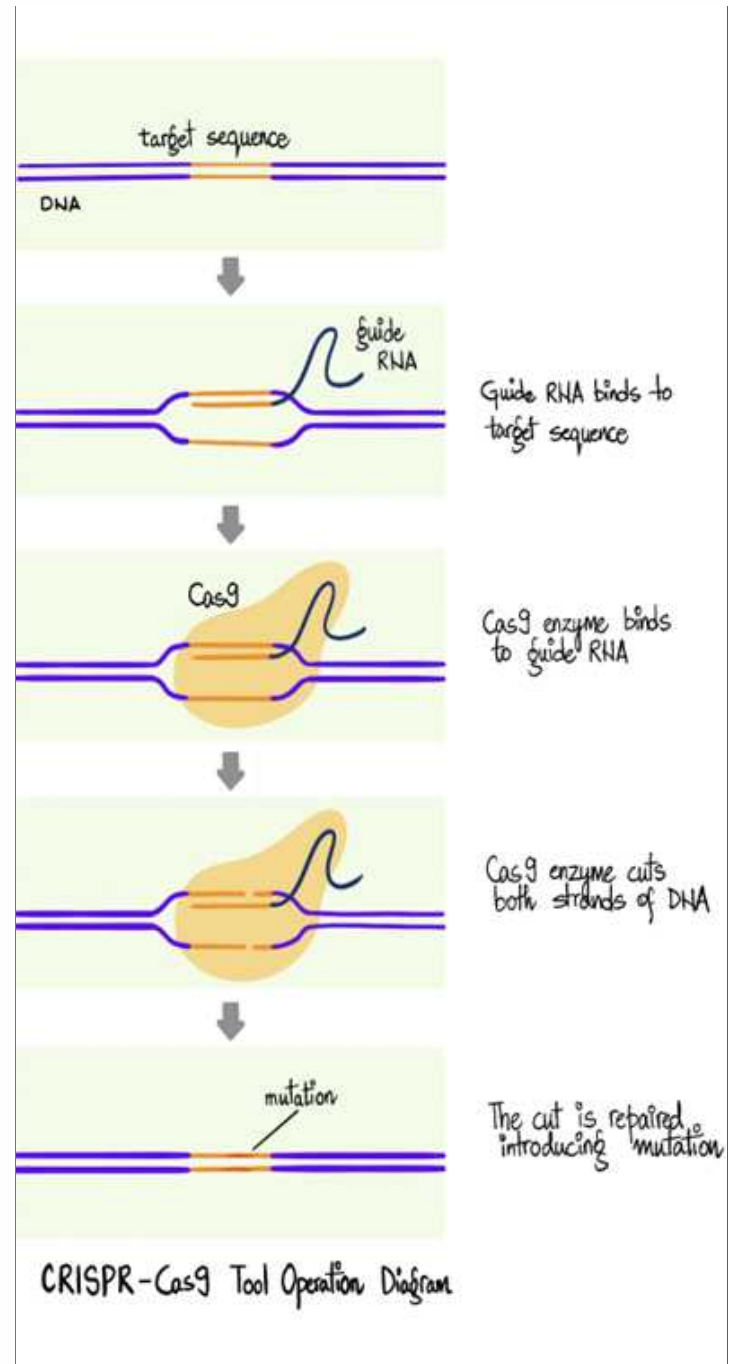
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Conversely, Cas9 is a nuclease that targets DNA by following short RNAs that have contrasting bases to Watson's. Compared to ZNFs and TALENs, CRISPR/Cas9 has shown to be a far more precise and effective genome editing technique in biological research. A successful utilization of the CRISPR/Cas9 framework has been demonstrated in the generation of novel varieties with improved features by targeting several genes in different crops.

The model from which CRISPR-Cas9 was derived is occurring naturally DNA editing process which bacteria used as immune response. Bacteria infected with virus grab hold of little bits of the virus DNA and rearrange it inside their DNA to create, what are known as CRISPR arrays. Bacteria can "remember" the viruses (or viruses which are closely related to them). In order to create RNA segments that can attach to particular DNA sequences on the viruses in the event that they launch another attack, the bacteria use CRISPR arrays. After then, the bacteria use an enzyme similar to Cas9 to split DNA, rendering the virus dysfunctional.

Working Technique?

CRISPR-Cas9 framework is composed of two fundamental parts which alter DNA. Out of these is an enzyme called Cas9. To enable the insertion or removal of DNA segments, which act as set of "molecular scissors" that can split the strands of DNA at a specific site in the genome. RNA segment also known as guide RNA or gRNA, it is composed of 20-base RNA sequence fragment that has been predesigned. It is situated within a bigger RNA structure. While the structural element binds to DNA, the pre-designed nucleotide "guides" Cas9 to the correct location within the genome. This guarantees that the Cas9 enzyme will cut the DNA at the precise spot. The guide RNA's job is to recognize and bind to a particular DNA sequence. Complementary RNA bases are present in both target DNA sequence of the genome and the guide RNA. It follows from this that, in theory, the guide RNA should only bind to the targeted region and not to any other regions of the genome [3]. Through the use of the DNA repair system, several genes throughout the genome of a target cell. Cas9 cleaves DNA strands at the exact location in the DNA sequence that the guide RNA indicates. At this juncture, cell attempts to repair the damaged DNA after realizing the extent to which damage has been done. Researchers are able to modify many genes within a target cell's entire genome by utilizing the DNA repair system.



The guide RNA typically has a specific 20-base arrangement. These complement the target sequence of the gene that needs to be changed. But for the gRNA to bind, not all 20 bases have to match. This causes an issue since a sequence that includes, let's say, 19 of 20 complementary nucleotides show up in another part of the genome altogether. This suggests that the target sequence might not be bound there, or the guide RNA may bind instead. The enzyme Cas9 will then cut at the incorrect spot, allowing a mutation to be inserted there. This mutation might not have any effect on the person at all, but it might affect an important gene or another significant component of the chromosome. Researchers are eager to discover a means to guarantee precise binding and cutting of CRISPR-Cas. This can be accomplished in two different manners: first, by using the knowledge of the genome's DNA sequence to create more precise guide RNAs and second, by taking into account the "off-target" behavior of various Cas9-gRNA complex types employing a Cas9 enzyme, which can only cut the target DNA's single strand as opposed to its double strand. This implies that for the cut to happen, two Cas9 enzymes and two guide RNAs must be present at the same location [4]. This lessens the chance of making incorrect cut.

Applications and Implications

Hepatitis B, cancer and hypercholesterolemia are just few of genetically based diseases for which CRISPR has substantial promise as a therapeutic approach. While editing reproductive tissues has caused a lot of attention, many of the applications that have been put out altering the genomes of somatic non-reproductive cells [8-12]. Since any modifications done to germline cells will be perpetuated by future generations, there are significant moral issues. In the UK and most other nations, it is currently illegal to change genes on germline cells. However, it is universally acknowledged that somatic cells are the only ones capable of utilizing CRISPR and other genome editing technologies.

Gene knockouts have been prior application of CRISPR technology in ornamentals. Additionally *Chrysanthemum morifolium*, *Petunia hybrida*, *Torenia*, *Ipomoea nil*, *Lilium longiflorum*, *Lilium pumilum*, *Dendrobium officinale* and *Phalaenopsis equestris* have all benefited from its successful use in generating gene knockouts in ornamental plants to induce genetic variations. According to these research, ornamental plants can effectively undergo mutagenesis produced by CRISPR/Cas9. Using the traditional Mendelian segregation, the modification generated is accurate and may be inherited by future generations (Sirohi et al. 2022).

CRISPR/Cas systems is easy, dependable and capable of multiplex targeting, they provide many benefits for the establishment in agricultural crops of resistance. Because of their high precision and efficiency, these systems hold considerable promise for overcoming the limits of conventional breeding for resistance development. CRISPR/Cas9 still has a lot of restrictions despite its many benefits and wide range of applications. Although S genes are linked to other desired genes, especially those that control plant growth and development, targeting them directly may have some fitness cost [5-7]. There are very few significant obstacles that could keep Cas9 technique from contributing significantly for the emergence of disease resistance. Furthermore, any interruption of the S gene may interfere with the product's pathway and eventually, the pathways of many additional products. Without taking into account species boundaries, most interesting plants can be edited to produce desirable S locus variants for selection. It is anticipated that more S genes will be identified, expanding the pool of possible genome modification targets. "Off target mutations" are a major constraint as well and are now a cornerstone of attempts to enhance the CRISPR system, especially in the transgene-free agricultural production process. Off-target genome editing is the term for DNA alterations at random and nonspecific locations that can happen via gRNA misguides or in a gRNA-independent way [8-14]. Beyond the technological difficulties of bringing CRISPR/Cas9-developed crops from lab to the field, other barriers include ambiguous legal frameworks, disagreements over intellectual property rights and acceptability by customers. A number of useful Cas9-based applied approaches have emerged that allow scientists to quickly improve plants. It should be mentioned, finally, that the CRISPR/Cas9 technique functions well for genetically modifying crops.

CONCLUSION

The application of CRISPR/Cas9 technology in floricultural crops marks a revolutionary leap forward in the quest for enhancing quality and productivity. The precision and efficiency offered by this genome editing tool provide unprecedented opportunities to address longstanding challenges in the floriculture industry. As we navigate the complexities of breeding and cultivation, CRISPR/Cas9 emerges as a powerful ally, offering targeted modifications that can elevate traits such as color, fragrance, and disease resistance.

One of the remarkable aspects of CRISPR/Cas9 is its potential to expedite the traditional breeding process, significantly reducing the time required to develop new varieties with desired characteristics. This acceleration not only meets the demands of an ever-evolving market but also allows for a more rapid response to emerging challenges, such as evolving pests and changing environmental conditions. Moreover, the ecological implications of CRISPR-edited floricultural crops cannot be

understated. By enhancing traits related to stress tolerance and pest resistance, we can envision a future where these crops contribute to sustainable and environmentally friendly agricultural practices. Reduced dependence on chemical inputs and improved resource use efficiency are promising outcomes that align with the broader goals of sustainable agriculture. However, as with any transformative technology, ethical considerations and regulatory frameworks must be carefully navigated. The responsible and transparent application of CRISPR/Cas9 in floricultural crops necessitates a collaborative effort involving researchers, policymakers, and industry stakeholders. Striking a balance between innovation and ethical responsibility will be key to ensuring public acceptance and realizing the full potential of this ground breaking technology. CRISPR/Cas9 technology with floriculture holds immense promise for advancing not only the economic viability of the industry but also its environmental sustainability. As we continue to unlock the potential of genetic editing tools, the blossoming future of floricultural crops appears more vibrant, resilient, and diverse than ever before. The seeds of innovation sown today have the potential to yield a harvest of beautiful and sustainable blooms that benefit both producers and consumers alike.

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